Instruction for use
A solid-phase enzyme immunoassay kit
for the quantitative determination of
thyroid microsomal antibodies in human serum or plasma

aTPO EIA

Catalogue number REF **K131**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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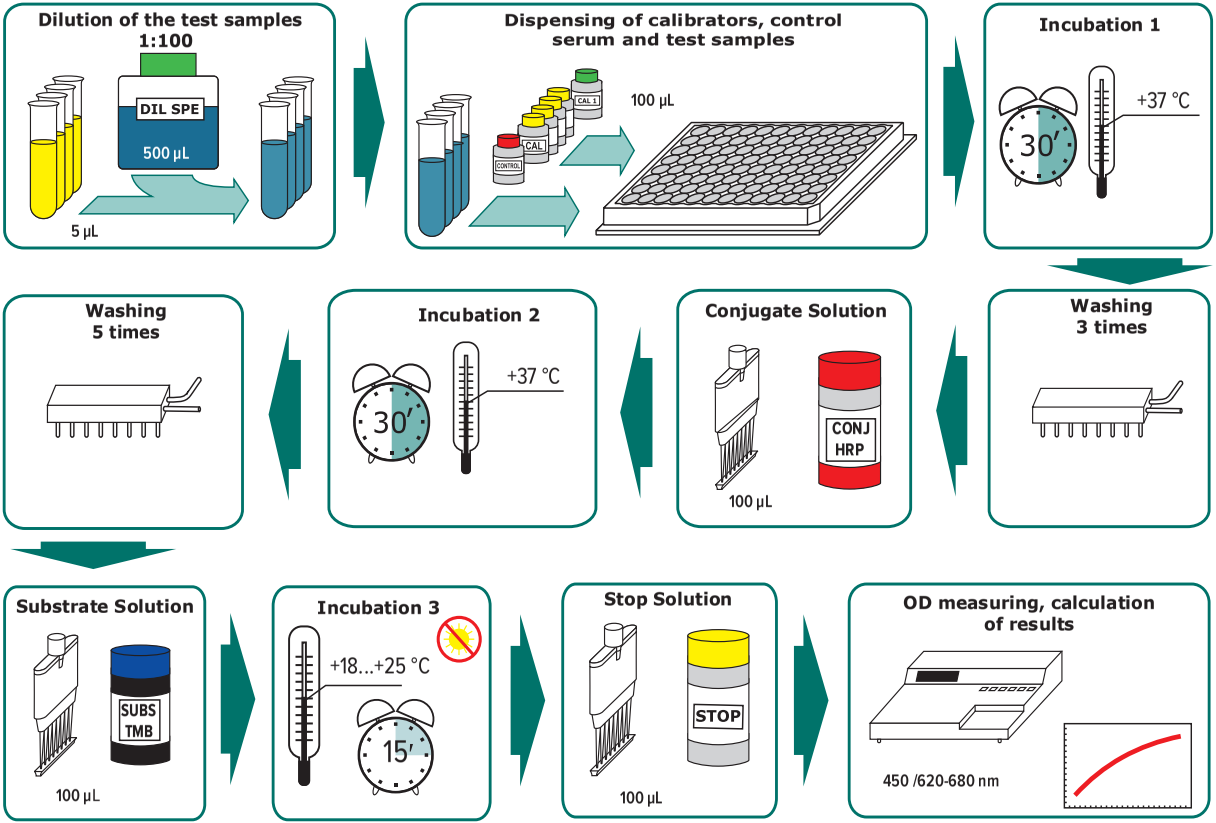
ASSAY PROCEDURE



K131

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of thyroid microsomal antibodies in human serum or plasma
aTPO EIA

1. INTENDED USE

The aTPO EIA kit is an enzyme immunoassay, intended for the quantitative determination of thyroid microsomal antibodies in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Anti-TPO antibodies (formerly – thyroid microsomal antibodies) are directed against a target protein – thyroid peroxidase (TPO) – located in the smooth endoplasmic reticulum of thyroid cells. The presence of anti-TPO antibodies in serum is associated with thyroid autoimmune diseases (Graves' disease and Hashimoto's thyroiditis). Anti-TPO antibodies mostly belong to the IgG class.

Low to moderate levels of serum anti-TPO antibodies can be found in some other autoimmune pathology (eg systemic lupus erythematosus or Sjogren syndrom) and, rarely, in apparently healthy subjects (especially elderly women). Anti-TPO antibodies are more sensitive in diagnosis of thyroid autoimmune diseases than anti-thyroglobulin (anti-TG) antibodies. However, in some cases anti-TG positive sera may be negative for anti-TPO. Therefore, combined determination of both types of anti-thyroid antibodies (anti-TPO + anti-TG) provides a more sensitive laboratory diagnostic tool for thyroid autoimmunity.

3. PRINCIPLE OF THE TEST

The determination of the anti-TPO antibodies (aTPO) is based on the indirect enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized antigen TPO. Second antibodies – murine monoclonal anti-IgG antibodies conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes three stages of incubation:

- during the first stage specific to antigen TPO antibodies from the specimen are bound by antigens coated onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated murine monoclonal antibodies bind to the antigen-antibody complexes, fixed in the formed at the previous stage complexes;
- during the third stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured specific autoantibodies to thyroperoxidase in test specimen.

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of anti-TPO antibodies in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P131Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with antigen TPO; ready to use
C131Z	CAL 1	**Calibrator C1**	1.1 mL	1	Solution based on phosphate buffer (pH 7.2-7.4), free of anti-TPO antibodies, with preservative, ready to use (colourless liquid)
C131Z	CAL 2-5	**Calibrators**	1.1 mL	4	Solutions based on phosphate buffer (pH 7.2-7.4), containing 30; 100; 300 and 1000 IU/mL of anti-TPO antibodies, with preservative, ready to use (red liquids)
Q131Z	CONTROL	**Control Serum**	1.1 mL	1	Solution based on human serum, containing of known anti-TPO antibodies content, with preservative, ready to use (colourless liquid)
T131Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of murine monocnoclonal antibodies to IgG conjugated to the horseradish peroxidase; ready to use (red liquid)
SP131Z	DIL SPE	**EIA Buffer**	50 mL	1	Buffer solution with detergent and preservative, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	30 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (3 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm or 450\620-680 nm wavelength;
- dry thermostat for +37°C±2°C;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- vortex mixer;
- disposable gloves;
- absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The aTPO EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The aTPO EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- EIA Buffer, Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing solution preparation

Add the contents of the 30 mL washing solution concentrate vial to 750 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the washing solution concentrate, mL	2.5	5	7.5	10	12.5	15	17.5	20	22.5	25	27.5	30
Volume of water, mL	62.5	125	187.5	250	312.5	375	437.5	500	562.5	625	687.5	750

9.4. Samples preparation

Dilute samples using EIA buffer 101 fold (for example, add to the vial 5 µL of the test sample + 500 µL EIA buffer).

If suggested analyte concentration in the sample exceeds the 1000 IU/mL, additionally dilute this sample accordingly, using EIA buffer. Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of biological fluids.

Do not dilute Control Serum and Calibrators!

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2 Dilute the test samples as described in 9.4.

10.3 Dispense **100 µL of Calibrators and Control Serum as well as 100 µL of diluted test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5	SAMP13	SAMP13						
D	CAL4	CAL4	SAMP6	SAMP6	SAMP14	SAMP14						
E	CAL5	CAL5	SAMP7	SAMP7	SAMP15	SAMP15						
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 3 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6 Add **100 µL of Conjugate Solution** to all wells.

10.7 Cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.8 At the end of the incubation period, aspirate and wash each well 5 times as described in 10.5.

10.9 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.10 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.11 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the stop solution. Set photometer blank on CAL1.

10.12 Plot a calibration curve in linear coordinates: (x) is the concentration of aTPO IU/mL in the calibrators, (y) – OD versus aTPO concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.13 Determine the corresponding concentration of aTPO in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

10.14 The aTPO EIA kit can be used for screening. For this purpose, it is necessary to add 100 µL of Calibrator CAL1 to the wells of the microplate in duplicates, and 100 µL of Calibrator CAL2 30 IU/mL to other wells in duplicates, to the rest wells - 100 µL of diluted tested samples. Compare the value of OD of each tested serum (plasma) sample with the OD of calibrator CAL2 30 IU/ml (IU/ml) (ODC). If the OD value of the test sample is higher than the ODC value (+10%), then the result should be considered as POSITIVE (more than 30 IU/ml aTPO). If the OD value of the tested sample is lower than the ODC value (-10%), then the result should be considered as NEGATIVE. If the OD value of the tested sample is within ± 10%, then this result should be considered EQUIVOCAL.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for aTPO. Based on data obtained by XEMA, the following normal range is recommended (see below).

NOTE: values of aTPO concentrations in the tested samples that are below the LoD (2.5 IU/mL) and also exceed the value of the upper calibrator (1000 IU/mL) should be provided in the following form: «the aTPO concentration of tested sample X is «lower than 2.5 IU/mL» or «higher than 1000 IU/mL».

Sex, age	Units, IU/mL	
	Lower limit	Upper limit
Males	-	30
Females	-	30
Females >50 yrs	-	50

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, IU/mL	CV, %
1	322.4	6.74
2	175.2	5.62

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, IU/mL	CV, %
1	341.6	7.15
2	181.7	4.48

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, IU/mL	Concentration2, IU/mL	Concentration3, IU/mL	CV, %
1	352.6	358.4	360.1	2.1
2	182.6	198.7	200.4	6.1

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known aTPO concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 30-300 IU/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest aTPO concentration in the serum or plasma sample that is detected by the aTPO EIA kit is no lower than 2.5 IU/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for aTPO EIA kit is 20 IU/mL.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

14. REFERENCES

1. Amino N., Mosi H., Iwatani W., Tanizawa O., Kawashima M., Tsuge I., Ibiragi K., Kumahara Y., Miyai K. – High prevalence of transient postpartum thyrotoxicosis and hypothyroidism. New Engl.J.Med., 1982, 306:84.
2. Bastenie P., Neve P., Bonnyns M., Van Haelts L., Chailly M. – Clinical and pathological significance of atrophic thyroiditis. Lancet, 1967, 1:915.
3. Bonnyns M., Van Haelts L., Bastenie P. – Asymptomatic atrophic thyroiditis. Horm. Res. 1982, 16:338.
4. Buchanan W., Alexander W., Crooks J., Koutras D., Wayne E., Anderson J.R., Goudie R. – Association of thyrotoxicosis and autoimmune thyroiditis. Brit.Med. J., 1961, 1:843.
5. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
6. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
7. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of autoantibodies to thyroglobulin in human serum or plasma

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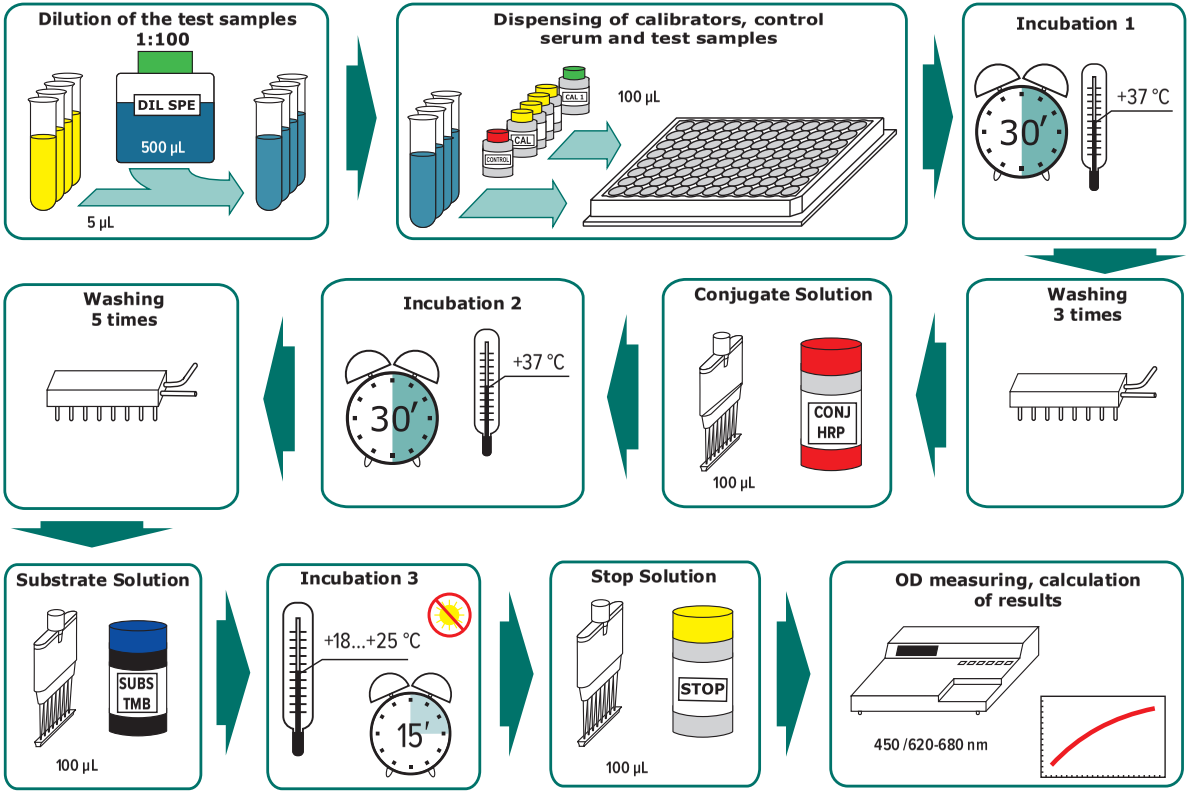
ASSAY PROCEDURE



K132

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of autoantibodies to thyroglobulin in human serum or plasma
aTG EIA

1. INTENDED USE

The aTG EIA kit is an enzyme immunoassay, intended for the quantitative determination of autoantibodies to thyroglobulin in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Thyroglobulin (TG) is a well known target for autoantibodies occurring in thyroid autoimmunity (Graves' disease and Hashimoto's thyroiditis). Anti-TG antibodies mostly belong to the IgG class. Low to moderate levels of anti-TG antibodies can be found in sera of other autoimmune patients (eg systemic lupus erythematosus or Sjogren syndrome).

In some cases anti-TG positive sera may show negativity for other type of anti-thyroid antibodies – anti-TPO. Therefore, combined determination of both types of anti-thyroid antibodies (anti-TPO + anti-TG) provides most sensitive laboratory diagnostic tool for thyroid autoimmunity. Separately from autoimmunity, anti-TG antibodies may develop in patients suffering from thyroid cancer. High level of anti-TG in such patients may interfere with correct determination of serum thyroglobulin which serves as tumour marker for therapy control in this group of patients.

3. PRINCIPLE OF THE TEST

The determination of the anti-TG antibodies (aTG) is based on the indirect enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized antigen Thyroglobulin. Second antibodies – murine monoclonal anti-IgG antibodies conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes three stages of incubation:

- during the first stage specific to antigen anti-TG antibodies from the specimen are bound by antigens coated onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated murine monoclonal antibodies bind to the antigen-antibody complexes, fixed in the formed at the previous stage complexes;
- during the third stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured specific autoantibodies to thyroglobulin in test specimen.

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of anti-TG antibodies in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P132Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with antigen Thyroglobulin; ready to use
C132Z	CAL 1	**Calibrator C1**	1.1 mL	1	Solution based on phosphate buffer (pH 7.2-7.4), free of anti-TG antibodies, with preservative, ready to use (colourless liquid)
C132Z	CAL 2-5	**Calibrators**	1.1 mL	4	Solutions based on phosphate buffer (pH 7.2-7.4), containing 100; 300; 1000 and 3000 IU/mL of anti-TG antibodies, with preservative, ready to use (blue liquids)
Q132Z	CONTROL	**Control Serum**	1.1 mL	1	Solution based on human serum, containing of known anti-TG antibodies content, with preservative, ready to use (colourless liquid)
T132Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of murine monocnoclonal antibodies to IgG conjugated to the horseradish peroxidase; ready to use (magenta liquid)
S011Z3	DIL	**EIA Buffer**	50 mL	1	Buffer solution with detergent and preservative, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	2	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (3 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm or 450\620-680 nm wavelength;
– dry thermostat for +37°C±1°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

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- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The aTG EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The aTG EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- EIA Buffer, Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing solution preparation

Add the contents of the 22 mL washing solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the washing solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

9.4. Samples preparation

Dilute samples using EIA buffer 101 fold (for example, add to the vial 5 µL of the test sample + 500 µL EIA buffer).

If suggested analyte concentration in the sample exceeds the 3000 IU/mL, additionally dilute this sample accordingly, using EIA buffer. Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of biological fluids.

Do not dilute Control Serum and Calibrators!

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2 Dilute the test samples as described in 9.4.

10.3 Dispense **100 µL of Calibrators and Control Serum as well as 100 µL of diluted test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5	SAMP13	SAMP13						
D	CAL4	CAL4	SAMP6	SAMP6	SAMP14	SAMP14						
E	CAL5	CAL5	SAMP7	SAMP7	SAMP15	SAMP15						
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 3 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6 Add **100 µL of Conjugate Solution** to all wells.

10.7 Cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.8 At the end of the incubation period, aspirate and wash each well 5 times as described in 10.5.

10.9 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18…+25°C) for 15 minutes**.

10.10 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.11 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the stop solution. Set photometer blank on CAL1.

10.12 Plot a calibration curve in linear coordinates: (x) is the concentration of aTG IU/mL in the calibrators, (y) – OD versus aTG concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.13 Determine the corresponding concentration of aTG in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for aTG. Based on data obtained by XEMA, the following normal range is recommended (see below).

NOTE: values of aTG concentrations in the tested samples that are below the LoD (5.0 IU/mL) and also exceed the value of the upper calibrator (3000 IU/mL) should be provided in the following form: «the aTG concentration of tested sample X is «lower than 5.0 IU/mL» or «higher than 3000 IU/mL».

Sex, age	Units, IU/mL	
	Lower limit	Upper limit
Males	-	100
Females	-	100
Females >50 yrs	-	150

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, IU/mL	CV, %
1	1256.9	2.46
2	110.7	5.39

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, IU/mL	CV, %
1	1264.5	4.33
2	107.9	6.43

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, IU/mL	Concentration2, IU/mL	Concentration3, IU/mL	CV, %
121	1270.5	1262.8	1276.6	0.54
433	109.4	114.5	118.5	4.00

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known aTG concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 100-3000 IU/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest aTG concentration in the serum or plasma sample that is detected by the aTG EIA kit is no lower than 5 IU/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for aTG EIA kit is 100 IU/mL.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

14. REFERENCES

1. U Feldt-Rasmussen – Analytical and clinical performance goals for testing autoantibodies to thyroperoxidase, thyroglobulin, and thyrotropin receptor. Clin. Chem., Jan 1996; 42: 160 – 163.
2. PW Ladenson – Optimal laboratory testing for diagnosis and monitoring of thyroid nodules, goiter, and thyroid cancer. Clin. Chem., Jan 1996; 42: 183 – 187.
3. Anthony P. Weetman – Graves' Disease. N. Engl. J. Med., Oct 2000; 343: 1236 – 1248.4. Buchanan W., Alexander W., Crooks J., Koutras D., Wayne E., Anderson J.R., Goudie R. – Association of thyrotoxicosis and autoimmune thyroiditis. Brit.Med. J., 1961, 1:843.
4. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
5. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
6. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

qa@xema.com.ua

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Instruction for use
A solid-phase enzyme immunoassay kit
for the quantitative determination of
IgG antibodies to tissue transglutaminase
in human serum or plasma

anti-TGlu IgG EIA

Catalogue number REF **K160**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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tel.:+ 49 911 931 639 67
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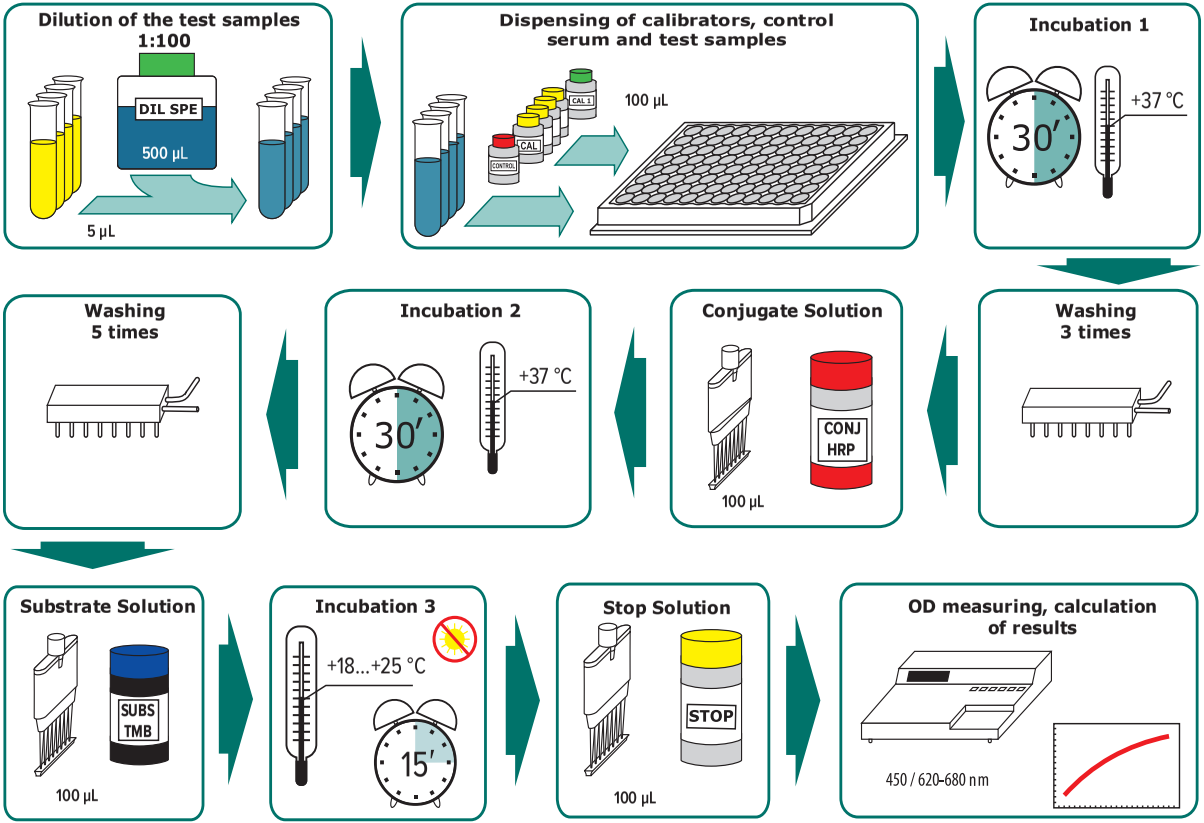
www.polmed.de

ASSAY PROCEDURE



K160

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of IgG antibodies to tissue transglutaminase in human serum or plasma anti-TGlu IgG EIA

1. INTENDED USE

The anti-TGlu IgG EIA kit is an enzyme immunoassay, intended for the quantitative determination of IgG antibodies to tissue transglutaminase in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Celiac disease (CD) or gluten-sensitive enteropathy is a chronic disease characterized by impaired intestinal absorption due to mucosal lesions. The exact etiology of CD is unknown but it is clearly shown that gliadin – the alcohol-soluble fraction of wheat gluten – is the toxic agent. Gliadin serves as a substrate for tissue Transglutaminase (TGlu) – a calcium-dependent enzyme constituent of the intestine mucosa. Gliadin-TGlu complex antigen induces the formation of IgA- and – later on – IgG-autoantibodies in patients with acute CD.

Previously, anti-TGlu antibodies were called «endomysium antibodies" and were detected by immunofluorescent methods on smooth muscle slides. After gluten exclusion from the diet, anti-TGlu antibody levels in the blood gradually decrease. To further confirm the diagnosis, a mucosal biopsy of the duodenal-jejunal junction is used, with characteristic lesions ("flat" mucosa) indicating the presence of severe/moderate CD. Thus, determination of anti-TGlu may be used for screening while mucosal biopsy – to confirm CD diagnosis.

Usually, CD onset occurs in early childhood after implementing additional feeding, but later on, the symptoms may spontaneously disappear notwithstanding continuing malabsorption. Nevertheless, even such mild pathology may lead to retardation of growth, puberty and even to dwarfness. Normally, following such a "remission", an onset of classic symptoms of CD occurs again during the 3rd-6th decades of life, and the correct diagnosis in such patients is made too late. Usually, mild and asymptomatic CD in adults manifests as unexplained anemia, hyposplenism, or osteoporosis.

It is rational (from the economical point of view as well) to screen the following patient groups for CD: children with growth retardation, unexplained anemia, unexplained hypocalcemia or osteomalacia, retardation of puberty, patients with insulin-dependent diabetes, persons having close relatives suffering from CD, patients with autoimmune thyroiditis, systemic connective tissue pathology, selective IgA deficiency.

3. PRINCIPLE OF THE TEST

The determination of IgG antibodies to TGlu is based on the indirect enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized antigen TGlu. Second antibodies – murine monoclonal anti-IgG antibodies conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes three stages of incubation:

- during the first stage specific to TGlu antibodies from the specimen are bound by antigens coated onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated murine monoclonal anti-IgG antibodies bind to the antigen-antibody complexes, fixed in the formed at the previous stage complexes;
- during the third stage, the complexes formed due to the reaction with the chromogen 3,3′,5,5′-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured specific IgG antibodies to TGlu in test specimen.

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of anti-TGlu IgG antibodies in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P160Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with antigen TGlu; ready to use
C160Z	CAL 1	**Calibrator C1**	1.1 mL	1	Solution based on phosphate buffer (pH 7.2-7.4), free of anti-TGlu IgG antibodies, with preservative, ready to use (colourless liquid)
C160Z	CAL 2-5	**Calibrators**	1.1 mL	4	Solutions based on phosphate buffer (pH 7.2-7.4), containing 25; 50; 100 and 200 U/mL of anti-TGlu IgG antibodies, with preservative, ready to use (red liquids)
Q160Z	CONTROL	**Control Serum**	1.1 mL	1	Solution based on human serum, containing of known content of anti-TGlu IgG antibodies, with preservative, ready to use (colourless liquid)
T160Z	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of murine monocnoclonal antibodies to IgG conjugated to the horseradish peroxidase; ready to use (red liquid)
SP160Z	DIL SPE	**EIA Buffer**	50 mL	1	Buffer solution with detergent and preservative, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	30 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm or 450\620-680 nm wavelength;
– dry thermostat for +37°C±1°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The anti-TGlu IgG EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The anti-TGlu IgG EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- EIA Buffer, Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing solution preparation

Add the contents of the 30 mL washing solution concentrate vial to 750 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the washing solution concentrate, mL	2.5	5	7.5	10	12.5	15	17.5	20	22.5	25	27.5	30
Volume of water, mL	62.5	125	187.5	250	312.5	375	437.5	500	562.5	625	687.5	750

9.4. Samples preparation

Dilute samples using EIA buffer 101 fold (for example, add to the vial 5 µL of the test sample + 500 µL EIA buffer).

If suggested analyte concentration in the sample exceeds the 200 U/mL, additionally dilute this sample accordingly, using EIA buffer. Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of biological fluids.

Do not dilute Control Serum and Calibrators!

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2 Dilute the test samples as described in 9.4.

10.3 Dispense **100 µL of Calibrators and Control Serum as well as 100 µL of diluted test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5	SAMP13	SAMP13						
E	CAL5	CAL5	SAMP6	SAMP6	SAMP14	SAMP14						
F	CAL6	CAL6	SAMP7	SAMP7	SAMP15	SAMP15						
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 3 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6 Add **100 µL of Conjugate Solution** to all wells.

10.7 Cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.8 At the end of the incubation period, aspirate and wash each well 5 times as described in 10.5.

10.9 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.10 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.11 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the stop solution. Set photometer blank on CAL1.

10.12 Plot a calibration curve in linear coordinates: (x) is the concentration of anti-TGlu IgG U/mL in the calibrators, (y) – OD versus anti-TGlu IgG concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. For the algorithm calculation (approximation) of the calibration curve, using the interval (segment-linear, point-to-point) method is recommended.

10.13 Determine the corresponding concentration of anti-TGlu IgG in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for anti-TGlu IgG. Based on data obtained by XEMA, the following normal range is recommended (see below).

NOTE: values of anti-TGlu IgG concentrations in the tested samples that are below the LoD (1.0 U/mL) and also exceed the value of the upper calibrator (200 U/mL) should be provided in the following form: «the anti-TGlu IgG concentration of tested sample X is «lower than 1.0 U/mL» or «higher than 200 U/mL».

Sex, age	Units, U/mL	
	Lower limit	Upper limit
Healthy donors	-	25.0

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, U/mL	CV, %
1	120.2	4.5
2	49.4	4.7

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, U/mL	CV, %
1	85.2	4.5
2	122.7	7.1

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, U/mL	Concentration2, U/mL	Concentration3, U/mL	CV, %
1	47.5	52.8	49.0	5.48
2	77.5	81.4	84.0	4.61

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known anti-TGlu IgG concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 10-100 U/mL±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest anti-TGlu IgG concentration in the serum or plasma sample that is detected by the anti-TGlu IgG EIA kit is no lower than 1.0 U/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for anti-TGlu IgG EIA kit is 5.0 U/mL.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

14. REFERENCES

1. Chartrand LJ, Seidman EG. Celiac disease is a lifelong disorder. Clin.Invest.Med., Vol. 19, 357-361, 1996

2. Cornell HJ. Coeliac disease: A review of the causative agents and their possible mechanisms of action. Amino Acids, Vol. 10, 1-19, 1996

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SAMPLES IDENTIFICATION PLAN

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	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

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Instruction for use
A solid-phase enzyme immunoassay kit
for the quantitative determination of
IgA antibodies to tissue transglutaminase
in human serum or plasma

anti-TGlu IgA EIA

Catalogue number REF **K161**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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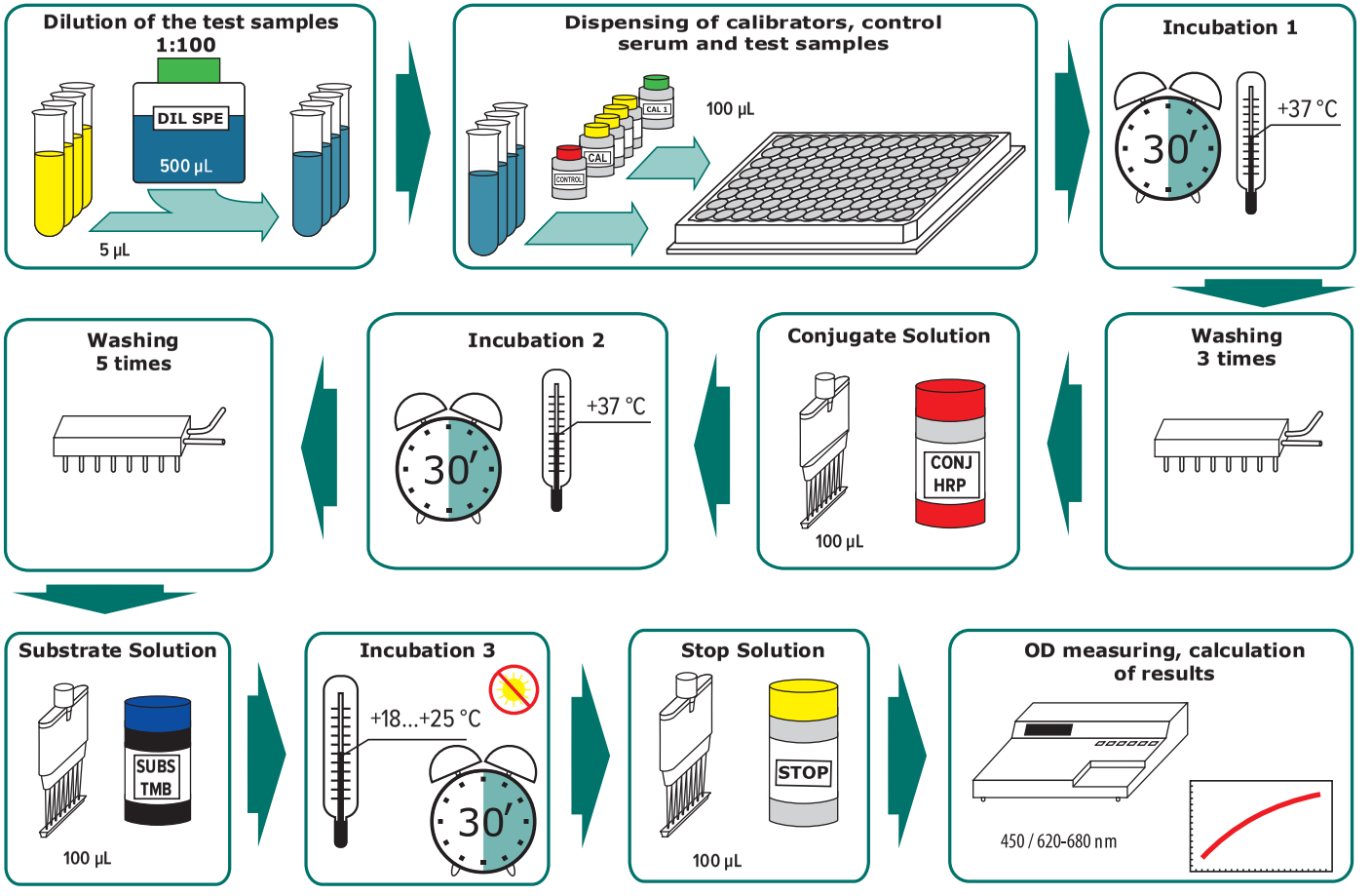
ASSAY PROCEDURE



K161

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of IgA antibodies to tissue transglutaminase in human serum or plasma
anti-TGlu IgA EIA

1. INTENDED USE

The anti-TGlu IgA EIA kit is an enzyme immunoassay, intended for the quantitative determination of IgA antibodies to tissue transglutaminase in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Celiac disease (CD) or gluten-sensitive enteropathy is a chronic disease characterized by impaired intestinal absorption due to mucosal lesions. The exact etiology of CD is unknown but it is clearly shown that gliadin – the alcohol-soluble fraction of wheat gluten – is the toxic agent. Gliadin serves as a substrate for tissue Transglutaminase (TGlu) – a calcium-dependent enzyme constituent of the intestine mucosa. Gliadin-TGlu complex antigen induces the formation of IgA- and – later on – IgG-autoantibodies in patients with acute CD.

Previously, anti-TGlu antibodies were called «endomysium antibodies" and were detected by immunofluorescent methods on smooth muscle slides. After gluten exclusion from the diet, anti-TGlu antibody levels in the blood gradually decrease. To further confirm the diagnosis, a mucosal biopsy of the duodenal-jejunal junction is used, with characteristic lesions ("flat" mucosa) indicating the presence of severe/moderate CD. Thus, determination of anti-TGlu may be used for screening while mucosal biopsy – to confirm CD diagnosis.

Usually, CD onset occurs in early childhood after implementing additional feeding, but later on, the symptoms may spontaneously disappear notwithstanding continuing malabsorption. Nevertheless, even such mild pathology may lead to retardation of growth, puberty and even to dwarfness. Normally, following such a "remission", an onset of classic symptoms of CD occurs again during the 3rd-6th decades of life, and the correct diagnosis in such patients is made too late. Usually, mild and asymptomatic CD in adults manifests as unexplained anemia, hyposplenism, or osteoporosis.

It is rational (from the economical point of view as well) to screen the following patient groups for CD: children with growth retardation, unexplained anemia, unexplained hypocalcemia or osteomalacia, retardation of puberty, patients with insulin-dependent diabetes, persons having close relatives suffering from CD, patients with autoimmune thyroiditis, systemic connective tissue pathology, selective IgA deficiency.

3. PRINCIPLE OF THE TEST

The determination of IgA antibodies to TGlu is based on the indirect enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized antigen TGlu. Second antibodies – murine monoclonal anti-IgA antibodies conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes three stages of incubation:

- during the first stage specific to TGlu antibodies from the specimen are bound by antigens coated onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated murine monoclonal anti-IgA antibodies bind to the antigen-antibody complexes, fixed in the formed at the previous stage complexes;
- during the third stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured specific IgA antibodies to TGlu in test specimen.

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of anti-TGlu IgA antibodies in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P160Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with antigen TGlu; ready to use
C161Z	CAL 1	**Calibrator C1**	1.1 mL	1	Solution based on phosphate buffer (pH 7.2-7.4), free of anti-TGlu IgA antibodies, with preservative, ready to use (colourless liquid)
C161Z	CAL 2-6	**Calibrators**	1.1 mL	5	Solutions based on phosphate buffer (pH 7.2-7.4), containing 10; 25; 50; 100 and 200 U/mL of anti-TGlu IgA antibodies, with preservative, ready to use (blue liquids)
Q161Z	CONTROL	**Control Serum**	1.1 mL	1	Solution based on human serum, containing of known content of anti-TGlu IgA antibodies, with preservative, ready to use (colourless liquid)
T161Z	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of murine monocnoclonal antibodies to IgA conjugated to the horseradish peroxidase; ready to use (blue liquid)
SP161Z	DIL SPE	**EIA Buffer**	50 mL	1	Buffer solution with detergent and preservative, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	30 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid, ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm or 450\620-680 nm wavelength;
– dry thermostat for +37°C±1°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The anti-TGlu IgA EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The anti-TGlu IgA EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- EIA Buffer, Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing solution preparation

Add the contents of the 30 mL washing solution concentrate vial to 750 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the washing solution concentrate, mL	2.5	5	7.5	10	12.5	15	17.5	20	22.5	25	27.5	30
Volume of water, mL	62.5	125	187.5	250	312.5	375	437.5	500	562.5	625	687.5	750

9.4. Samples preparation

Dilute samples using EIA buffer 101 fold (for example, add to the vial 5 µL of the test sample + 500 µL EIA buffer).

If suggested analyte concentration in the sample exceeds the 200 U/mL, additionally dilute this sample accordingly, using EIA buffer. Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of biological fluids.

Do not dilute Control Serum and Calibrators!

10. ASSAY PROCEDURE

10.1. Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 14 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-6) and 2 wells for Control Serum (Q)).

10.2. Dilute the test samples as described in 9.4.

10.3. Dispense **100 µL of Calibrators and Control Serum as well as 100 µL of diluted test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5	SAMP13	SAMP13						
E	CAL5	CAL5	SAMP6	SAMP6	SAMP14	SAMP14						
F	CAL6	CAL6	SAMP7	SAMP7	SAMP15	SAMP15						
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.4. Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.5. At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 3 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6. Add **100 µL of Conjugate Solution** to all wells.

10.7. Cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.8. At the end of the incubation period, aspirate and wash each well 5 times as described in 10.5.

10.9. Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 30 minutes**.

10.10. Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.11. Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the stop solution.

10.12. Plot a calibration curve in linear coordinates: (x) is the concentration of anti-TGlu IgA U/mL in the calibrators, (y) – OD versus anti-TGlu IgA concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. For the algorithm calculation (approximation) of the calibration curve, using the interval (segment-linear, point-to-point) method is recommended.

10.13. Determine the corresponding concentration of anti-TGlu IgA in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for anti-TGlu IgA. Based on data obtained by XEMA, the following normal range is recommended (see below).

NOTE: values of anti-TGlu IgA concentrations in the tested samples that are below the LoD (1.0 U/mL) and also exceed the value of the upper calibrator (200 U/mL) should be provided in the following form: «the anti-TGlu IgA concentration of tested sample X is «lower than 1.0 U/mL» or «higher than 200 U/mL».

Sex, age	Units, U/mL	
	Lower limit	Upper limit
Healthy donors	-	20

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1. Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, U/mL	CV, %
1	43.2	3.5
2	31.4	6.9

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, U/mL	CV, %
1	27.3	6.17
2	48.1	3.7

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, U/mL	Concentration2, U/mL	Concentration3, U/mL	CV, %
1	32.5	33.8	31.0	7.48
2	66.1	64.8	67.3	6.61

13.1.2. Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known anti-TGlu IgA concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 10-100 U/mL±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest anti-TGlu IgA concentration in the serum or plasma sample that is detected by the anti-TGlu IgA EIA kit is no lower than 1.0 U/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for anti-TGlu IgA EIA kit is 5.0 U/mL.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

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LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

qa@xema.com.ua

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of total IgE in human serum or plasma

Total IgE EIA

Catalogue number REF **K200**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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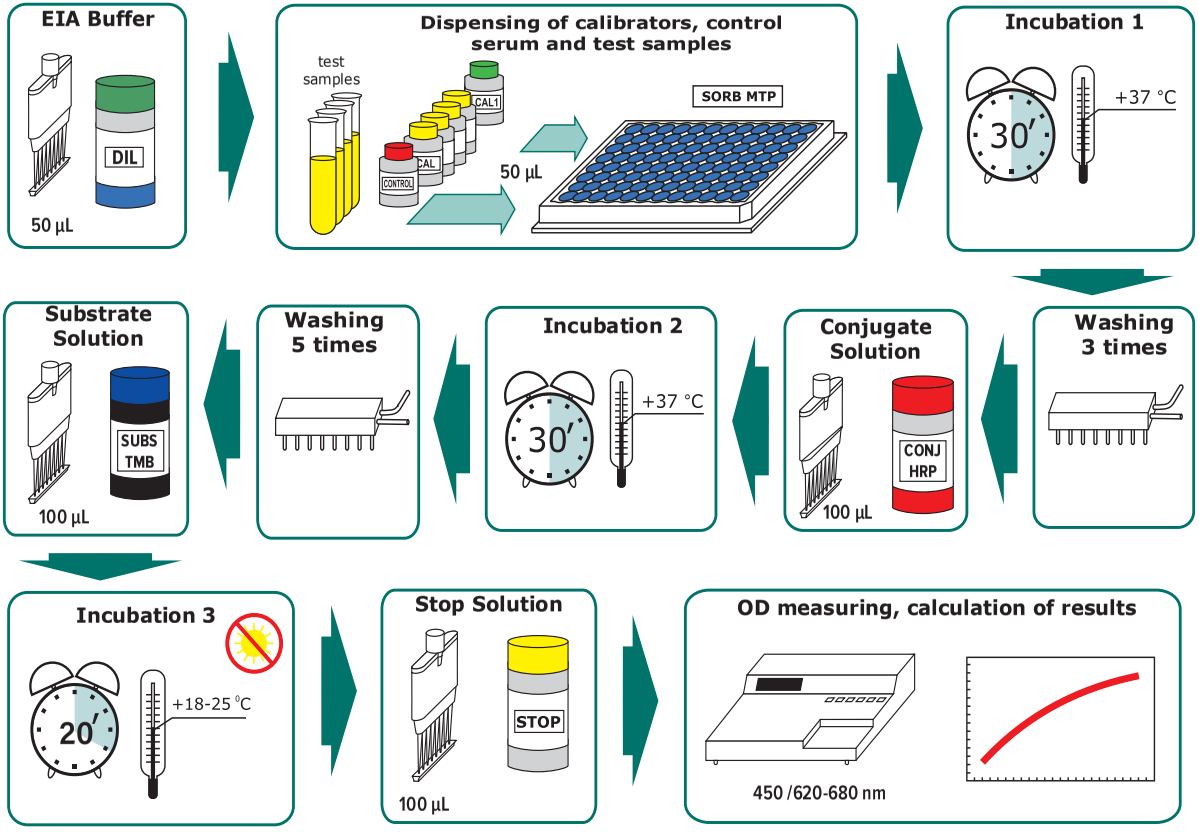
ASSAY PROCEDURE



K200

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of total IgE in human serum or plasma
Total IgE EIA

1. INTENDED USE

The Total IgE EIA kit is an enzyme immunoassay, intended for the quantitative determination of total IgE concentration in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Total immunoglobulin E (IgE) serum level is widely reported as the laboratory marker of atopic diseases such as atopic asthma, atopic dermatitis, and pollenosis. An atopic (IgE-dependent) mechanism can also underlie gastroenterocolitis, urticaria, other forms of vasculitis (including systemic), cholecystitis, vulvovaginitis, and cystitis. Part of the drug allergy (mainly to penicillin and protein drugs) also develops according to the IgE-dependent mechanism. In all of the conditions listed above, the production of high titers of specific IgE antibodies can lead to an increase in the level of total IgE in the serum. A particularly high level of total IgE is characteristic of atopic dermatitis. In addition to atopic diseases, total serum IgE is significantly increased in parasitic infestations and mycoses (especially systemic), rarely in systemic autoimmune diseases and immunodeficiency states (especially in hyper-IgE syndrome), as well as in mastocytosis (mast cell tumor) and extremely rare IgE-myeloma. A decrease in the level of total IgE in serum (below 15 IU/ml in adults) is a rare and little-studied phenomenon described in hypogammaglobulinemia, some autoimmune diseases, ulcerative colitis, and primary biliary cirrhosis.

3. TEST PRINCIPLE

The determination of the total IgE is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to human IgE. Second antibodies – rabbit polyclonal antibodies to IgE conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes tree stages of incubation:

- during the first stage the total IgE from the specimen is captured by the monoclonal antibodies coated onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated with rabbit polyclonal antibodies bind to free epitopes of immobilized total IgE, fixed in the formed at the previous stage complexes;
- during the third stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured total IgE in the serum specimen (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of total IgE in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P200Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to total IgE; ready to use
C200Z	CAL 1	**Calibrator C1**	0.8 mL	1	Solution based on phosphate buffer, free of total IgE, with preservative, ready to use (yellow liquid)
C200Z	CAL 1-5	**Calibrators**	0.8 mL	5	Solutions based on phosphate buffer, containing 50; 200; 500 and 1000 IU/mL of total IgE, ready to use (red liquids)
Q200Z	CONTROL	**Control serum**	0.8 mL	1	Solution based on human serum, containing of known total IgE content, with preservative, ready to use (colourless liquid)
T200Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of rabbit polyclonal antibodies to human total IgE conjugated to the horseradish peroxidase; ready to use (red liquid)
S011Z	DIL	**EIA Buffer**	14 mL	1	Buffer solution with detergent and preservative, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	30 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (3 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm wavelength or 450\620-680 nm;
- dry thermostat for 37 °C±2 °C;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- vortex mixer;
- disposable gloves;
- absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The Total IgE EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The Total IgE EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- EIA Buffer, Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing solution preparation

Add the contents of the 30 mL washing solution concentrate vial to 750 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the washing solution concentrate, mL	2.5	5	7.5	10	12.5	15	17.5	20	22.5	25	27.5	30
Volume of water, mL	62.5	125	187.5	250	312.5	375	437.5	500	562.5	625	687.5	750

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2 Dispense **50 µL of EIA Buffer** to all wells.

10.3 Dispense **50 µL of Calibrators and Control Serum as well as 50 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5	SAMP13	SAMP13						
D	CAL4	CAL4	SAMP6	SAMP6	SAMP14	SAMP14						
E	CAL5	CAL5	SAMP7	SAMP7	SAMP15	SAMP15						
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **30 minutes at +37 °C**.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 3 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6 Add **100 µL of Conjugate Solution** to all wells.

10.7 Cover strips with a plate sealing tape and incubate for **30 minutes at +37 °C**.

10.8 At the end of the incubation period, aspirate and wash each well 5 times as described in 10.5.

10.9 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 20 minutes**.

10.10 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.11 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the stop solution. Set photometer blank on CAL1.

10.12 Plot a calibration curve in linear coordinates: (x) is the concentration of total IgE in the Calibrators IU/mL, (y) – OD versus concentration of total IgE (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.13 Determine the corresponding concentration of total IgE in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

12.1. Therapeutical consequences should not be based on the results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for total IgE. Based on data obtained by XEMA LLC, the following normal range is recommended (see below).

NOTE: values of total IgE concentrations in the tested samples that are below the LoD (3 IU/mL) and also exceed the value of the upper calibrator (1000 IU/mL) should be provided in the following form : «the total IgE concentration of tested sample X is «lower than 3 IU/mL» or «higher than 1000 IU/mL».

12.2. The calibrators concentration values of the Total IgE EIA kit are expressed in IU/mL. To calculate concentrations in ng/mL, the received concentration value in IU/mL shall be multiplied by 2.4.

1 IU/mL = 2.4 ng/mL.

Sex, age	Units, IU/mL		Units alternative, ng/mL	
	Lower limit	Upper limit	Lower limit	Upper limit
< 6 months	-	12	-	28.8
6-12 months	-	30	-	72.0
1-3 yrs	-	45	-	108.0
4-6 yrs	-	70	-	168.0
7-9 yrs	-	90	-	216.0
10-15 yrs	-	120	-	288.0
>15 yrs	-	130	-	312.0

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, IU/mL	CV, %
1	10.6	4.33
2	116.2	5.47

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, IU/mL	CV, %
1	12.5	8.36
2	113.4	1.47

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, IU/mL	Concentration2, IU/mL	Concentration3, IU/mL	CV, %
1	12.7	13.3	12.3	3.66
2	115.5	117.8	115.1	1.25

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known total IgE concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 50-1000 IU/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest total IgE concentration in the serum or plasma sample that is detected by the Total IgE EIA kit is no lower than 3 IU/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for Total IgE EIA kit is 50IU/mL.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of total IgE with other analytes is shown in the table:

Analyte	Concentration, IU/mL	Cross-reactivity, %
IgA	1000	Not detected
IgM	1000	Not detected
IgG	1000	Not detected

14. REFERENCES

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2. Buckley R. H. Immunopharmacology of Allergic Disease 1979; 117.
3. Michel f. B., Bousquet J. and Greilier P. J. Allergy Clin. Immunol. 1980; 64:422.
4. Ishizaka T. Ann Allergy 1982; 48: 313.
5. Kulczyski A. Jr. J. Allergy Clin. Immunol. 1981; 68:5.
6. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
7. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
8. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
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LOT _____ DATE _____

XEMA

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
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For any issues related to operation of the kit and technical support, please contact by telefon number

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of thyroid stimulating hormone in human serum or plasma

TSH EIA

Catalogue number REF **K201**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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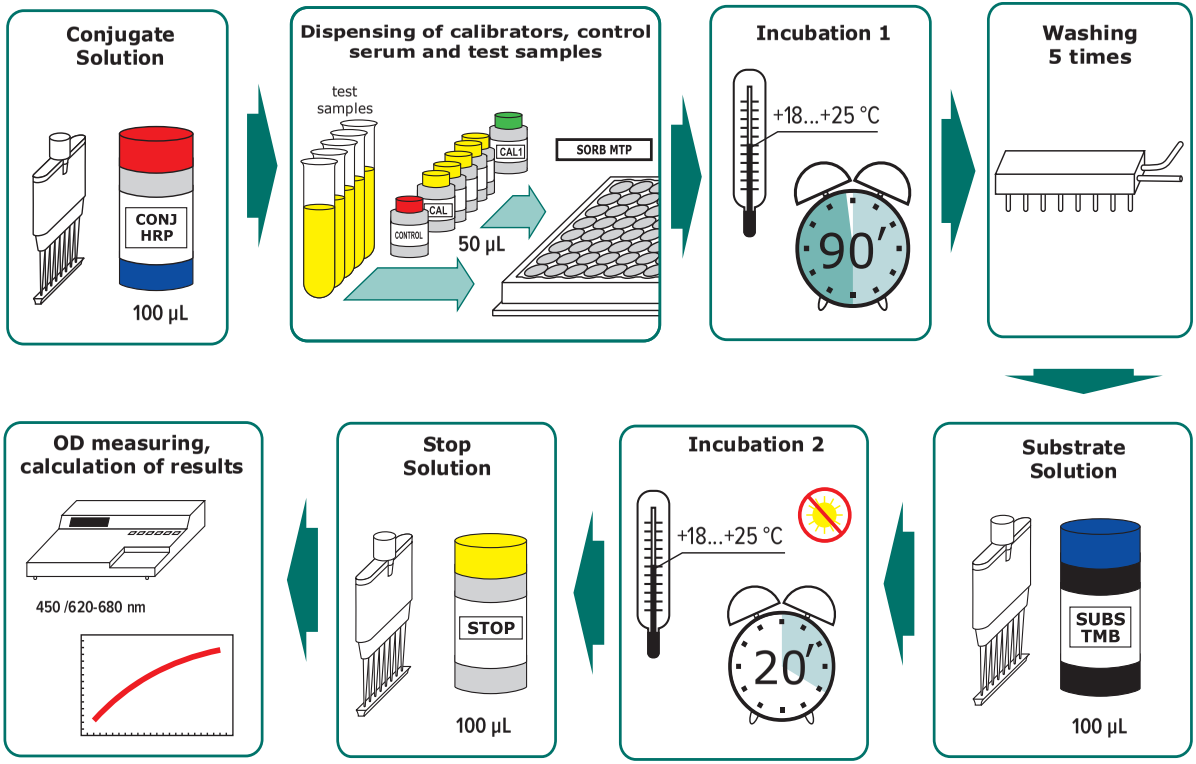
ASSAY PROCEDURE



K201

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of thyroid stimulating hormone in human serum or plasma
TSH EIA

1. INTENDED USE

The TSH EIA kit is an enzyme immunoassay, intended for the quantitative determination of thyroid stimulating hormone in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Thyroid stimulating hormone (TSH) is a glycoprotein with molecular weight ca.30 kDa which is secreted by hypophysis. A molecule of TSH consists of two noncovalently bound subunits: α and β. β-subunit determines biological activity and immunological specificity of TSH.

TSH stimulates thyroid gland to secrete thyroid hormones. When the concentration of these hormones in blood serum increases secretion of TSH is inhibited; on the contrary, when the level of thyroid hormones decreases, in the pituitary gland, the release of TSH increases, and therefore the production and release increases thyroid hormones.TSH secretion is subject to circadian rhythms with highest levels seen early in the morning (6 a.m.). Changes of TSH blood level during a day are not significant; nevertheless, if the results do not correspond with clinical status and other laboratory data, it is recommended to take and test another blood sample.

Determination of TSH level in serum is recommended in the following states and conditions:

1) diagnostics of dysfunction of the thyroid gland;

2) hypothyroidism (TSH level is increased. The diagnosis is confirmed by low concentrations of total and free T4 and T3. In mild subclinical forms when T4 and T3 levels are within normal ranges, determination of TSH concentration is critical);

3) hyperthyroidism (synthesis and secretion of TSH are inhibited); monitoring of replacement therapy;

4) screening for congenital hypothyroidism (on the fifth day of life, the level is determined TSH in a blood spot on filter paper or in blood serum). TSH level elevated at birth (up to 35 mIU/L), but after a few days it decreases to basal (both in boys and in girls).

Serum TSH level is elevated during pregnancy, after physical stress, in individuals with lowered blood pressure and lowered temperature. Secretion of TSH is inhibited by Cortisol and Growth hormone. Low TSH levels are often seen in elderly people, in patients with chronic renal insufficiency, liver cirrhosis, in retardation of sexual development, in secondary amenorrhea, Cushing syndrome, acromegaly.

3. PRINCIPLE OF THE TEST

The determination of TSH is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to β-chain of human TSH. Second antibodies – Fab 2 fragment of murine monoclonal antibodies to human TSH conjugated to the horseradish peroxidase is used as enzyme conjugate.

The analysis procedure includes two stages of incubation:

- during the first stage TSH from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized TSH;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured TSH in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density on the content of TSH in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P201Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to β-chain of human TSH; ready to use
C201Z	CAL 1	**Calibrator C1**	2 mL	1	Solution based on phosphate buffer (pH 7.2-7.4), free of human TSH, with preservative, ready to use (yellow liquid)
C201Z	CAL 2-6	**Calibrators**	0.8 mL	5	Solution based on phosphate buffer (pH 7.2-7.4), containing 0,2; 1; 5; 10 and 20 mIU/L of human TSH, with preservative, ready to use (red liquids)
Q201Z	CONTROL	**Control Serum**	0.8 mL	1	Solution based on human serum, containing of known human TSH content, with preservative, ready to use (colourless liquid)
T201Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of Fab 2 fragment of murine monoclonal antibodies to human TSH conjugated to the horseradish peroxidase; ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength or 450\620-680 nm;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The TSH EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The TSH EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months.

  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

  Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 20 mIU/L, additionally dilute this sample accordingly, using (Calibrator C1). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of the blood serum (plasma) sample

Do not dilute Control Serum and Calibrators!

10. ПРОВЕДЕННЯ АНАЛІЗУ

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 14 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-6) and 2 wells for Control Serum (Q)).

10.2 If necessary, dilute the test samples as described in 9.4.

10.3 Dispense **100 µL of Conjugate Solution** to all wells.

10.4 Dispense **50 µL of Calibrators and Control Serum as well as 50 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5								
E	CAL5	CAL5	SAMP6	SAMP6								
F	CAL6	CAL6	SAMP7	SAMP7								
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.5 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **90 minutes at room temperature (+18...+25°C)**.

10.6 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the wash solution volume can be increased to 350 µL.

10.7 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 20 minutes**.

10.8 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.9 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the Stop Solution. Set photometer blank on CAL1.

10.10 Plot a calibration curve in linear coordinates: (x) is the TSH concentration in the calibrators mIU/L, (y) – OD versus TSH concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.11 Determine the corresponding concentration of TSH in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on the results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for TSH. Based on data obtained by XEMA, the following normal range is recommended (see below).

NOTE: values of TSH concentrations in the tested samples that are below the LoD (0.04 mIU/L) and also exceed the value of the upper Calibrator (20 mIU/L) should be provided in the following form : «the TSH concentration of tested sample X is «lower than 0.04 mIU/L» or «higher than 20 mIU/L».

Sex, age	Units, mIU/L	
	Lower limit	Upper limit
Healthy donors	0.3	4.0

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, mIU/L	CV, %
1	2.12	7.2
2	3.64	3.8

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, mIU/L	CV, %
1	2.27	12.0
2	3.87	6.4

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, mIU/L	Concentration2, mIU/L	Concentration3, mIU/L	CV, %
1	2.32	2.02	1.81	9.9
2	3.71	3.56	3.32	5.6

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known TSH concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 0.2-10 mIU/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest TSH concentration in the serum or plasma sample that is detected by the TSH EIA kit is no lower than 0.04 mIU/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for TSH EIA kit is 0,15 mIU/L.

13.1.5 Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 20 mIU/L.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of TSH with other analytes is shown in the table:

Analyte	Cross-reactivity, %
HCG	< 0.1
LH	< 0.1
FSH	< 0.1

14. REFERENCES

1. Ekins R. Methods for measurement of free thyroid hormones. In: Free thyroid hormones. Amsterdam: Expecta Medica; 1979; p. 72-92.
2. Tietz, N., Fundamentals of Clinical Chemistry, W.B. Saunders Co., Philadelphia: 791 and 844 (1976).
3. Soos, M., Taylor, S.J., Gard, T., and Siddle, K.A. Rapid Sensitive Two-Site Immunometric Assay for TSH Using Monoclonal Antibodies: Investigation of Factors Affecting Optimisation. J. of Immunological Methods 73, 237-249 (1984).
4. Lundberg, P. A., Jagenburg, R., Lindstedt, G., Nystrom, E., Clin. Chem. 1982, 28:1241.
5. Musto, J.D., Pizzolante, J.M., Chesarone, V.P. A Comment of Thyrotropin Measurement and Evaluation. Clin. Chem. 30, 329-330 (1984). Opinion.
6. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
7. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
8. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

qa@xema.com.ua

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www.xema.in.ua

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of luteinizing hormone in human serum or plasma

LH EIA

Catalogue number REF **K202**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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tel.:+ 49 911 931 639 67
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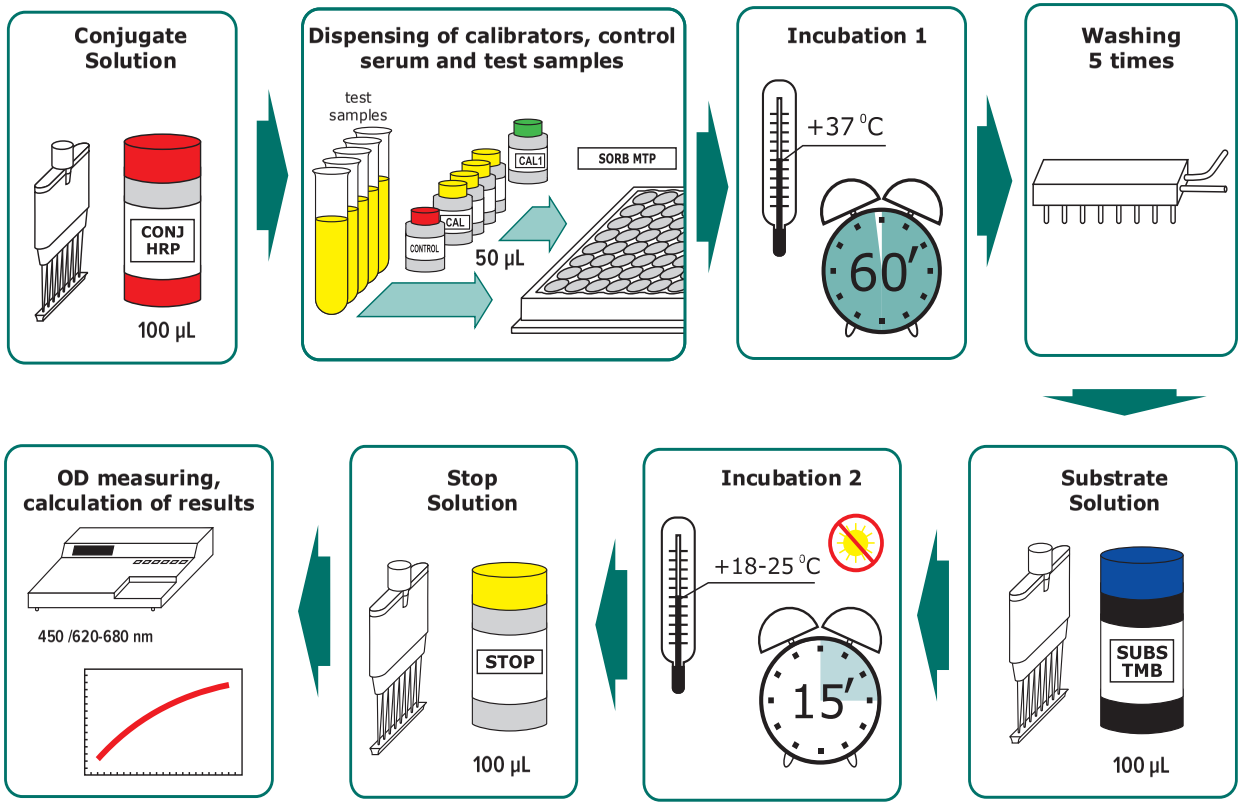
ASSAY PROCEDURE



K202

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of luteinizing hormone in human serum or plasma
LH EIA

1. INTENDED USE

The LH EIA kit is an enzyme immunoassay, intended for the quantitative determination of luteinizing hormone in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Luteinizing hormone (LH) is produced in both men and women by the anterior pituitary gland in response to luteinizing hormone-releasing hormone (LH-RH or Gn-RH), which is released by the hypothalamus. LH, also called interstitial cellstimulating hormone (ICSH) in men, is a glycoprotein with a molecular weight of approximately 30,000 daltons. It is composed of two noncovalently associated amino acid chains: alpha and beta.

The basal secretion of LH in men is episodic and has the primary function of stimulating the interstitial cells (Leydig cells) to produce testosterone. The variation in LH concentrations in women is subject to the complex ovulatory cycle of healthy menstruating women and depends on the sequence of hormonal events along the gonadohypothalamus-pituitary axis. During the cycle, LH level is low except for the middle of the cycle when its concentration may increase up to 5–10 fold. LH peak is preceded by a peak of Estradiol which occurs approximately 12 hours earlier. Ovulation occurs 12-120 hrs after LH peak. When the ovum is released, the corpus luteum is formed which secretes progesterone and estradiol, these latter exerting negative feedback effects on LH and FSH levels through hypothalamo-pituitary axis.

LH concentration in blood is subject to circadian rhythms; therefore blood samples for LH assay should always be taken at the same time of the day. Circadian variations of LH level are more pronounced in women depending on the stage of the menstrual cycle: they become less frequent at the end of the lutein phase and less pronounced – at the end of the follicular stage. Increased LH levels are found in primary dysfunction of gonadal glands, in amenorrhea caused by ovarian insufficiency, in Stein-Leventhal syndrome, after menopause. Increased concentrations of LH are also present during renal failure, cirrhosis, hyperthyroidism, and severe starvation.

Decreased LH concentrations are seen in dysfunction of hypophysis or hypothalamus, in galactorrhea-amenorrhea syndrome, in isolated decrease of gonadotropins, in the isolated LH decrease; in neurotic anorexia, in patients with retardation of growth and sexual development, after intake of digoxin, phenothiazine, progesterone, estrogens.

In the differential diagnosis of hypothalamic, pituitary, or gonadal dysfunction, assays of LH concentration are routinely performed in conjugation with FSH assays since their roles are closely interrelated. Furthermore, the hormone levels are used to determine menopause, pinpoint ovulation, and monitor endocrine therapy.

3. PRINCIPLE OF THE TEST

The determination of LH is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to β-chain of human LH. Second antibodies – Fab 2 fragment of murine monoclonal antibodies to α-chain human LH/FSH/HCG conjugated to the horseradish peroxidase is used as enzyme conjugate.

The analysis procedure includes two stages of incubation:

- during the first stage LH from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized α-chain human LH/FSH/HCG;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3’,5,5’-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured LH in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density on the content of LH in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P202Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to β-chain of human LH; ready to use
C202Z	CAL 1	**Calibrator C1**	2 mL	1	Solution based on human serum free of human LH, with preservative, ready to use (colourless liquid)
C202Z	CAL 2-5	**Calibrators**	0.6 mL	4	Solutions based on human serum, containing 5; 25; 50; 100 IU/L of human LH, with preservative, ready to use (red liquids)
Q202Z	CONTROL	**Control Serum**	0.6 mL	1	Solution based on human serum, containing of known human LH content, with preservative, ready to use (colourless liquid)
T202Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of Fab 2 fragment of murine monoclonal antibodies to α-chain human LH/FSH/HCG conjugated to the horseradish peroxidase; ready to use (red liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength or 450\620-680 nm;
– dry thermostat for 37 °C±1 °C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The LH EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The LH EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months.

  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

  Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 100 IU/L, additionally dilute this sample accordingly, using (Calibrator C1). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of the blood serum (plasma) sample.

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2 If necessary, dilute the test samples as described in 9.4.

10.3 Dispense **100 µL of Conjugate Solution** to all wells.

10.4 Dispense **50 µL of Calibrators and Control Serum as well as 50 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5								
D	CAL4	CAL4	SAMP6	SAMP6								
E	CAL5	CAL5	SAMP7	SAMP7								
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.5 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.6 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the wash solution volume can be increased to 350 µL.

10.7 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.8 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.9 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the Stop Solution. Set photometer blank on CAL1.

10.10 Plot a calibration curve in linear coordinates: (x) is the LH concentration in the calibrators IU/L, (y) – OD versus LH concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.11 Determine the corresponding concentration of LH in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for LH. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

NOTE: values of LH concentrations in the tested samples that are below the LoD (0.15 IU/L) and also exceed the value of the upper Calibrator (100 IU/L) should be provided in the following form : «the LH concentration of tested sample X is «lower than 0.15 IU/L» or «higher than 100IU/L».

Sex, age	Units, IU/L	
	Lower limit	Upper limit
Children under 11 yrs	1.0	5.0
Males	1.5	9.0
Females		
Menstrual cycle:		
follicular phase	2.0	9.5
ovulation	10.0	45
luteinic phase	0.5	17
post menopausal	5.0	57

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, IU/L	CV, %
1	4.96	6.2
2	16.41	3.9

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, IU/L	CV, %
1	4.87	10.0
2	16.01	5.4

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, IU/L	Concentration2, IU/L	Concentration3, IU/L	CV, %
1	4,94	4,83	5,0	1,75
2	16,3	16,56	16,01	1,69

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known LH concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 5-100 IU/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest LH concentration in the serum or plasma sample that is detected by the LH EIA kit is no lower than 0.15 IU/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for LH EIA kit is 5 IU/L.

13.1.5 Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 100 IU/L.

13.1.6 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of LH with other analytes is shown in the table:

Analyte	Cross-reactivity, %
HCG	< 0.1
TSH	< 0.1
FSH	< 0.1

14. REFERENCES

1. Pierce, J.G. and Parsons, T.F. Glycoprotein hormones: Structure and Function, Annual Rev. Biochem., 50, 465-495 (1981).
2. Harris, G.W. and Naftolinf., The Hypothalamus and Control of Ovulation, Brit. Med. Bullet., 26, 1-9 (1970).
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4. Jeffcoate, S.L., Clinics in Endocrinol. Metab., 4, 521-543 (1975).
5. Whitely, R.J., Keutmann, H.T. and Ryan, R.J., Endocrinology, 102, 1874 (1978).
6. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
7. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
8. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
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LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of follicle stimulating hormone in human serum or plasma

FSH EIA

Catalogue number REF **K203**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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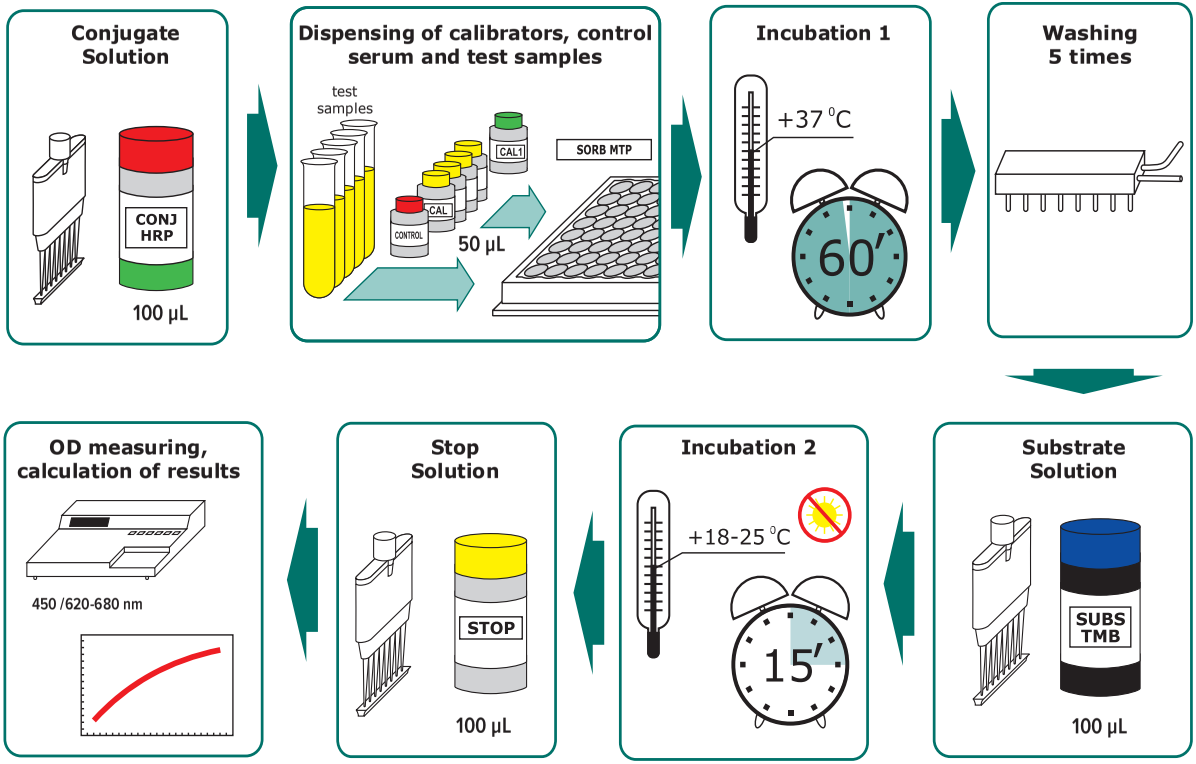
ASSAY PROCEDURE



K203

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of follicle stimulating hormone in human serum or plasma
FSH EIA

1. INTENDED USE

The FSH EIA kit is an enzyme immunoassay, intended for the quantitative determination of follicle stimulating hormone in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Follicle stimulating hormone (FSH) is a glycoprotein with molecular weight 28 kDa secreted by basophil cells in hypophysis. Gonadotropin releasing hormone (GnRH) produced by the hypothalamus controls the release of FSH from anterior pituitary. Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) are intimately involved in the control of the growth and reproductive activities of the gonadal tissues, which synthesize and secrete male and female sex hormones. The levels of circulating FSH and LH are controlled by these sex hormones through a negative feedback. Like LH, TSH and HCG, FSH consists of two subunits – alpha and beta, its biological and immunological properties being dependent on the hormone-specific beta subunit.

In females, FSH stimulates the growth and maturation of ovarian follicles. At the beginning of normal menstrual cycle FSH level is higher that at the final stage of follicular phase. Peak FSH levels are seen in the middle of the cycle concomitantly with LH peak levels. Increased estradiol and progesterone production during luteinic phase leads to decreased FSH blood concentrations by negative feedback mechanism. The same mechanism leads to elevation of FSH levels at the end of the cycle due to decreased estrogen and progesterone concentrations, and the new cycle is initiated.

In men, FSH regulates growth of seminiferous tubules and maintenance of spermatogenesis. However, androgens, unlike estrogen, do not lower FSH level, therefore demonstrating a feedback relationship only with serum LH. High levels of FSH in women are seen in menopause, preliminary ovarian failure, agenesia of ovaries; in men elevated FSH levels may be found in primary testicular failure, dysgenesia of seminiferous tubules, delayed sexual maturation, and Klinefelter syndrome. Elevated concentrations are also found in cases of starvation, renal failure, hyperthyroidism, cirrhosis and after intake of clomifen, l-DOPA.

Decreased FSH levels are found in hypopituitarism and after intake of oral contraceptives, phenotiazine, estrogens.

3. PRINCIPLE OF THE TEST

The determination of FSH is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to β-chain of human FSH. Second antibodies – murine monoclonal antibodies to α-chain human LH/FSH/HCG conjugated to the horseradish peroxidase is used as enzyme conjugate.

The analysis procedure includes two stages of incubation:

- during the first stage FSH from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized α-chain human LH/FSH/HCG;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured FSH in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density on the content of FSH in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P203Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to β-chain of human FSH; ready to use
C203Z	CAL 1	**Calibrator C1**	2 mL	1	PSolution based on human serum free of human FSH, with preservative, ready to use (colourless liquid)
C203Z	CAL 2-5	**Calibrators**	0.8 mL	4	Solutions based on human serum, containing 5; 25; 50; 100 IU/L of human FSH, with preservative, ready to use (green liquids)
Q203Z	CONTROL	**Control Serum**	0.8 mL	1	Solution based on human serum, containing of known human FSH content, with preservative, ready to use (colourless liquid)
T203Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of murine monoclonal antibodies to α-chain human LH/FSH/HCG conjugated to the horseradish peroxidase; ready to use (green liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength or 450\620-680 nm;
– dry thermostat for 37 °C±2 °C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The FSH EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The FSH EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months.
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 100 IU/L, additionally dilute this sample accordingly, using (Calibrator C1). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of the blood serum (plasma) sample.

10. ПРОВЕДЕННЯ АНАЛІЗУ

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2 If necessary, dilute the test samples as described in 9.4.

10.3 Dispense **100 µL of Conjugate Solution** to all wells.

10.4 Dispense **50 µL of Calibrators and Control Serum as well as 50 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5								
D	CAL4	CAL4	SAMP6	SAMP6								
E	CAL5	CAL5	SAMP7	SAMP7								
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.5 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.6 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the wash solution volume can be increased to 350 µL.

10.7 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.8 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.9 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the Stop Solution. Set photometer blank on CAL1.

10.10 Plot a calibration curve in linear coordinates: (x) is the FSH concentration in the calibrators IU/L, (y) – OD versus FSH concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.11 Determine the corresponding concentration of FSH in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for FSH. Based on data obtained by XEMA, the following normal range is recommended (see below).

NOTE: values of FSH concentrations in the tested samples that are below the LoD (0.15 IU/L) and also exceed the value of the upper Calibrator (100 IU/L) should be provided in the following form : «the FSH concentration of tested sample X is «lower than 0.15 IU/L» or «higher than 100 IU/L».

Sex, age	Units, IU/L	
	Lower limit	Upper limit
Children under 11 yrs	-	4.0
Males	0.8	25.0
Females		
Menstrual cycle:		
follicular phase	3.0	12
ovulation	2.0	12
luteinic phase	6.0	25
post menopausal	10.0	150

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, IU/L	CV, %
1	18.76	6.69
2	6.51	7.29

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, IU/L	CV, %
1	9,28	7,16
2	13,78	7,28

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, IU/L	Concentration2, IU/L	Concentration3, IU/L	CV, %
1	8,32	8,77	7,81	8,6
2	12,34	12,56	12,00	6,7

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known FSH concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 5-50 IU/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest FSH concentration in the serum or plasma sample that is detected by the FSH EIA kit is no lower than 0.15 IU/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for FSH EIA kit is 2.5 IU/L.

13.1.5 Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 100 IU/L.

13.1.6 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of FSH with other analytes is shown in the table:

Analyte	Cross-reactivity, %
HCG	< 0.1
TSH	< 0.1
LH	< 0.1

14. REFERENCES

1. Ross, F. T., Vande Wiele, R. L. and Franty, A. G.: Text of Endocrinol., Chapter 7, Ed.: R. H. Williams,W .B. Saunders, Philadelphia (1981).
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8. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

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LOT _____ DATE _____

XEMA

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
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LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of human chorionic gonadotropin in human serum or plasma

hCG EIA

Catalogue number REF **K205**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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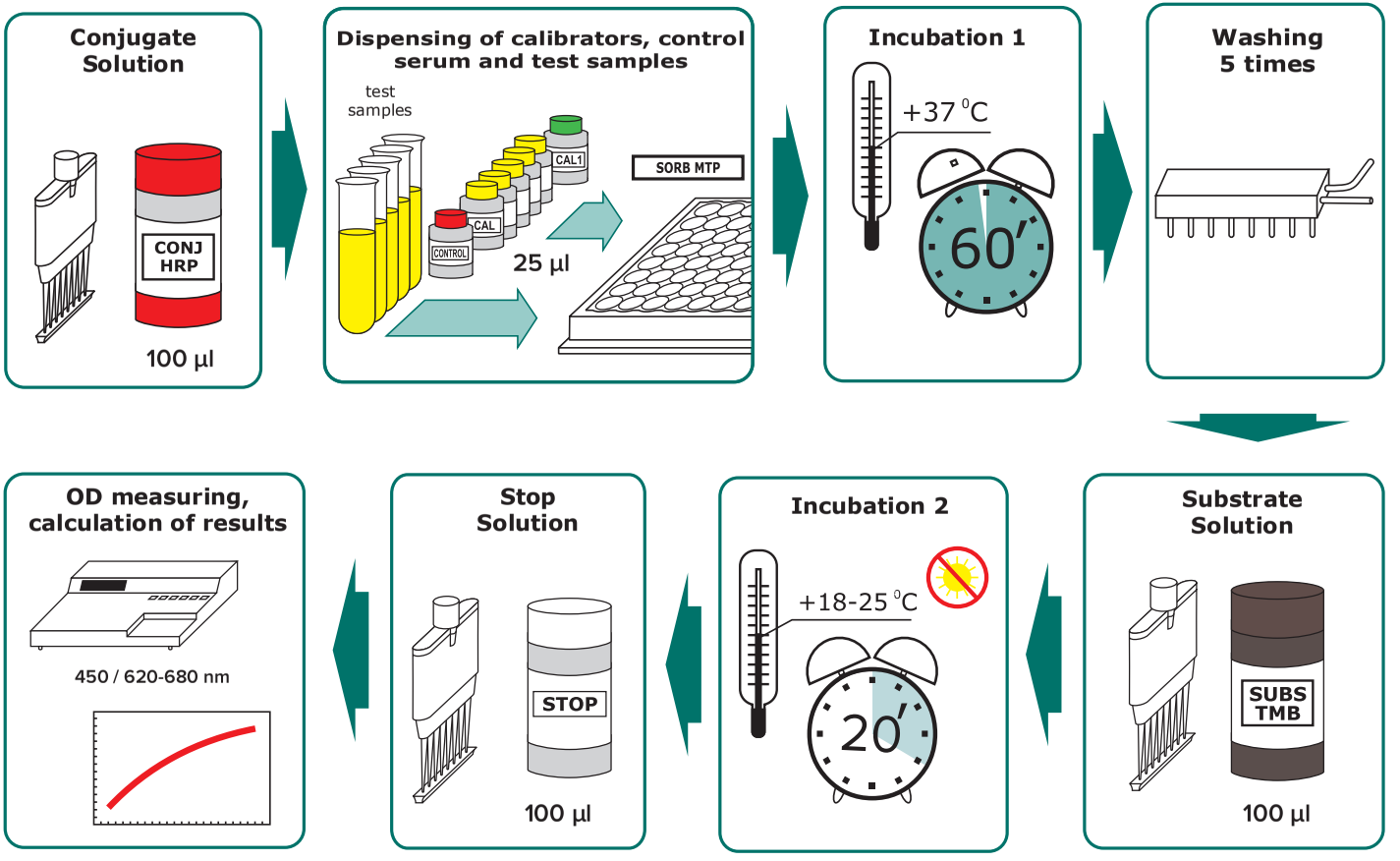
ASSAY PROCEDURE



During performing several independent series of tests, Calibrators and Control Serum should be used **each time**.

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of human chorionic gonadotropin in human serum or plasma

hCG EIA

1. INTENDED USE

The hCG EIA kit is an enzyme immunoassay, intended for the quantitative determination of human chorionic gonadotropin in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Chorionic gonadotropin (CG) is a glycoprotein secreted by trophoblastic cells of the placenta. The hCG molecule consists of two non-covalently linked polypeptide chains: α- and β-subunits. The α-subunit of hCG is not unique and is completely identical to the α-subunit of luteinising, follicle-stimulating and thyroid-stimulating hormones of the pituitary gland. The β-subunit is 80% homologous in structure to the β-chain of luteinising hormone, but has distinctive and special biological functions. The specificity and biological activity of the hormone is determined by its β-subunit.

The synthesis of hCG is mainly carried out by the syncytial layer of the trophoblast during pregnancy. The majority of circulating hCG is metabolised by liver cells, with about 20% excreted in the urine. The hormone maintains the activity and existence of the corpus luteum, taking over this role from luteinising hormone 6-8 days after ovulation. It is the main hormone of early pregnancy and stimulates the development of the trophoblast. Normally, during pregnancy between the 2nd and 5th week, the amount of beta-hCG doubles every 1.5 days. The concentration of hCG in urine and serum is determined for early diagnosis of pregnancy. In multiple pregnancies, serum hCG levels are significantly higher than normal for the period of pregnancy. Below-normal levels of the hormone at different stages of fetal development suggest ectopic pregnancy, fetal delay, threat of spontaneous abortion, non-developing pregnancy, or placental dysfunction. The determination of serum hCG in the second trimester of pregnancy (along with AFP and estriol) is included in the screening programme for Down's syndrome. In addition, hCG is the main laboratory diagnostic marker of chorionic epithelioma and other trophoblastic tumours and is a good indicator of the effectiveness of anticancer therapy.

Determining the level of hCG is part of a study that can be used to judge some fetal abnormalities, which allows a woman to be placed in a risk group for this pathology.

The hCG ELISA kit uses a monoclonal antibody specific to the β-subunit as a capture antibody; detection of the bound antigen is performed using a monoclonal antibody specific to the α-subunit; thus, only the intact hCG molecule is detected.

3. TEST PRINCIPLE

The determination of chorionic gonadotropin in blood serum (plasma) is based on the principle of a two-site «sandwich» version of a solid-phase enzyme-linked immunosorbent assay. Mouse monoclonal antibodies to the β-subunit of human chorionic gonadotropin are immobilised on the inner surface of the plate wells. Horseradish peroxidase conjugated mouse monoclonal antibodies to the α-chain of human LH/FSH/hCG are used as a conjugate. The assay procedure includes two stages of incubation:

- during the first stage, the hCG in the test sample binds to specific monoclonal antibodies on the surface of the well, and the horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of the immobilised hCG;
- during the second stage, the formed complexes are visualised as a result of the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured hCG in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density on the content of hCG in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P205Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to β chain hCG, ready to use
C205Z	CAL 1	**Calibrator C1**	0.6 mL	1	Solution based on phosphate buffer (pH 7.2-7.4), free of hCG, with preservative, ready to use (colourless or yellow liquid)
C205Z	CAL 2-6	**Calibrators**	0.6 mL	5	Solutions based on phosphate buffer (pH 7.2-7.4), containing 15; 60; 125; 250 and 500 IU/L of hCG, with preservative, ready to use (blue liquids)
Q205Z	CONTROL	**Control Serum**	0.6 mL	1	Solution based on human serum, containing of known hCG content, with preservative, ready to use (colourless or yellow liquid)
T205Z	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of murine monocnoclonal antibodies to α chain of hCG conjugated to the horseradish peroxidase, ready to use (red liquid)
S011Z4	DIL	**EIA Buffer**	100 mL	1	Buffer solution with detergent and preservative, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution, ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid, ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (1 pcs.)					

K205IE

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450/620-680 nm wavelength;
– dry thermostat for 37°C±1°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The hCG EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The hCG EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, EIA Buffer and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

9.4. Samples preparation

Depending on the week of gestation or if the estimated hCG concentration in the specimen is greater than 500 IU/L, the specimen must be further diluted using EIA Buffer (S011Z4). Use of other buffers and reagents for sample dilution may result in erroneous results.

Do not dilute Control Serum and Calibrators!

Mandatory dilutions of blood serum (plasma) samples are required depending on the week of pregnancy:

1-2 weeks - no dilution;
3-4 weeks - 1:20;
4-14 weeks - 1:400; 1:1000;
14-21 weeks - 1:400; 1:1000;
22 week, 3rd trimester - 1:400.

Example of dilution of blood serum (plasma) samples:
1:20 – 25 µL of the test sample + 475 µL of EIA buffer;
1:400 – 25 µL of 1:20 dilution + 475 µL EIA buffer;
1:1000 - 200 µL dilution 1:400 + 300 µL EIA buffer.

10. ASSAY PROCEDURE

10.1. Put the desired number of strips into the frame based on the number of test samples and 14 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-6) and 2 wells for Control Serum (Q)).

10.2. If necessary, dilute the test samples as described in 9.4.

10.3. Dispense **100 µL of Conjugate Solution** to all wells.

10.4. Dispense **25 µL of Calibrators and Control Serum as well as 25 µL of the diluted sample** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

During performing several independent series of tests, Calibrators and Control Sample should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5								
E	CAL5	CAL5	SAMP6	SAMP6								
F	CAL6	CAL6	SAMP7	SAMP7								
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.5. Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.6. At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well **5 times** using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5 µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the wash solution volume can be increased to 350 µL.

10.7. Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 20 minutes**.
The incubation time can be varied depending on the intensity of the blue colour development.

10.8. Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.9. Read the optical density (OD) of the wells at 450 nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.10. Plot a calibration curve in linear coordinates: (x) is the hCG concentration in the calibrators IU/L , (y) – OD versus hCG concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. For the algorithm calculation (approximation) of the calibration curve, using the interval (segment-linear, point-to-point) method is recommended.

10.11. Determine the corresponding concentration of hCG in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is below 0.15, the OD of CAL6 is above the critical value (see Quality control Data Sheet) and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

12.1. Therapeutical consequences should not be based on the results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for hCG. Based on data obtained by XEMA LLC, the following normal range is recommended (see below).

NOTE: values of hCG concentrations in the tested samples that are below the LoQ (10 IU/L) should be provided in the following form: «the hCG concentration of tested sample X is lower than 10 IU/L». If values of hCG concentrations in the tested samples exceed the value of the upper Calibrator (500 IU/L) they must be further diluted and tested.

12.2. The calibrators concentration values of the hCG EIA kit are expressed in IU/L. To calculate concentrations in ng/mL, the received concentration value in IU/L shall be multiplied by 0.2.

1 IU/L = 0.2 ng/mL

Sex, age	Units, IU/L		Units alternative, ng/mL	
	Lower limit	Upper limit	Lower limit	Upper limit
Males	-	15	-	3.0
Females	-	15	-	3.0
Pregnancy week:				
1	-	50	-	10.0
2	20	500	4.0	100
3	500	5000	100	1000
4	3000	19000	600	3800
5-8	14000	169000	2800	33800
9-13	16000	180000	3200	36000
22	4500	70000	900	14000
23	3000	69500	600	13900
3rd trimestr	2400	50000	480	10000

Medians and SD (recommended normal range 0.5-2.0)

Pregnancy, week	Median, kIU/L	SD
14	55	0.83
15	42.8	0.78
16	38.4	0.74
17	32.8	0.75
18	25.6	0.69
19	23	0.56
20	21	0.52
21	18	0.51

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1. Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, IU/L	CV, %
1	25.46	3.72
2	113.4	6.43

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, IU/L	CV, %
1	26.33	8.4
2	115.25	2.4

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, IU/L	Concentration2, IU/L	Concentration3, IU/L	CV, %
1	25.37	27.46	26.52	4.0
2	114.17	116.34	115.98	1.0

13.1.2. Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3. Linearity

Linearity was determined using sera samples with known hCG concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 10–250 IU/L ±10%.

13.1.4. Analytical sensitivity

Limit of detection (LoD) – the lowest hCG concentration in the serum or plasma sample that is detected by the hCG EIA kit is no lower than 1.25 IU/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for hCG EIA kit is 10 IU/L.

13.1.5. Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 500 IU/L.

13.1.6. Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of hCG with other analytes is shown in the table:

Analyte	Concentration	Cross-reactivity, %
LH	300 mIU/mL	9
	200 mIU/mL	< 5
	80 mIU/mL	< 5
FSH	75 µIU/mL	10
	50 µIU/mL	6
	25 µIU/mL	<5
TSH	200 mIU/mL	<5
	50 mIU/mL	<5

13.1.7. Metrological traceability

The concentrations of the calibration samples of the hCG - ELISA reagent kit comply with the WHO International Standard 6th International Standard for Chorionic Gonadotrophin, human, code NIBSC: 18/244.

14. REFERENCES

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5. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
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7. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81).

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

K205IE

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
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For any issues related to operation of the kit and technical support, please contact by telefon number

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of prolactin in human serum or plasma

Prolactin EIA

Catalogue number REF **K206**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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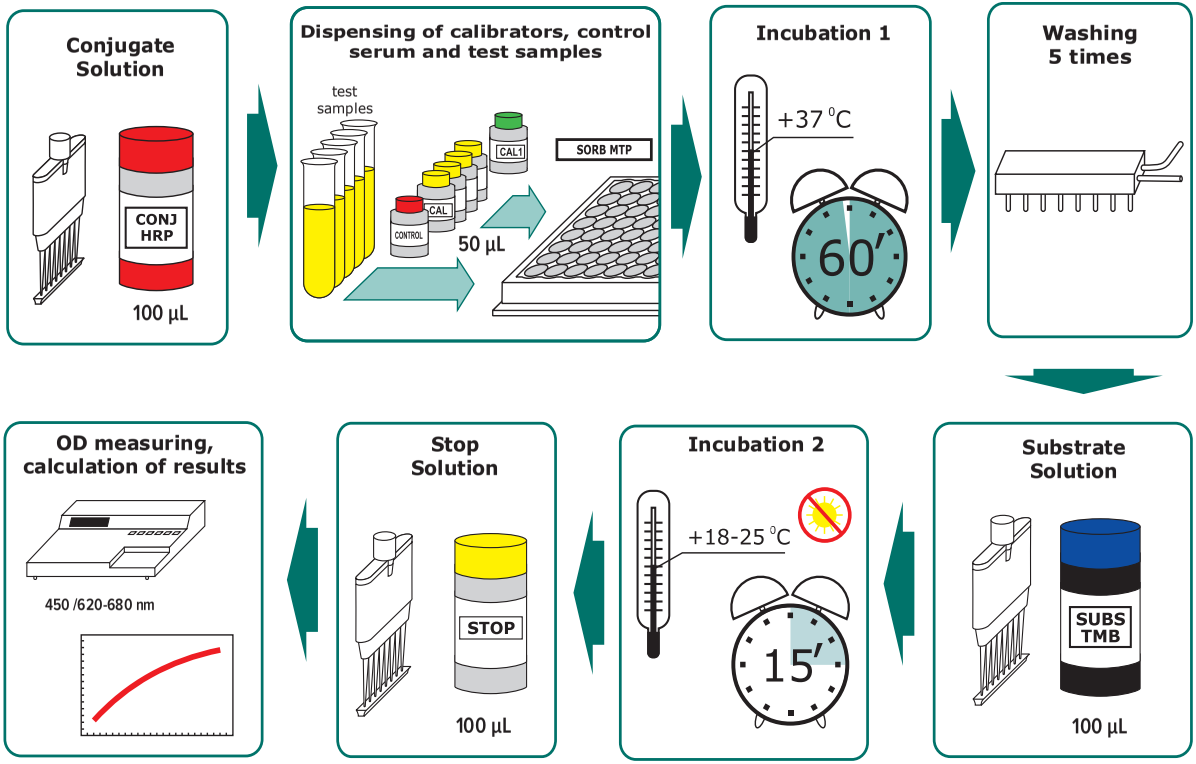
ASSAY PROCEDURE



K206

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of prolactin in human serum or plasma
Prolactin EIA

1. INTENDED USE

The Prolactin EIA kit is an enzyme immunoassay, intended for the quantitative determination of prolactin in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Prolactin is a 198 aminoacids polypeptide with a molecular weight of ca. 22.5 kDa which is secreted by eosinophil cells of hypophysis.

Hyperplasia and adenomas of hypophysis are the main causes of infertility. Functional changes in the regulation of reproductory function are also caused by alterations in the secretion of hormones of hypophysis. One of the markers of such alterations is changes in Prolactin secretion. That is why the WHO recommended to use determination of Prolactin level as a screening test in the primary laboratory investigation of couples claiming infertility.

In women, the Prolactin level remains low before menarche and elevates during puberty. During this period, Prolactin stimulates the development of mammary glands. Prolactin level changes during the menstrual cycle with elevations up to 900 mIU/l seen during periovulatory period and the second stage of luteinic phase. That is why it is recommended to evaluate the Prolactin level during the first stage of the cycle. Besides, physiological hyperprolactinemia is seen in stress conditions and after physical exercises.

Prolactin secretion is subject to circadian rhythms with maximal levels found during the night (3-7 fold higher than during the day). That is why the time of sampling is extremely important.

Elevated Prolactin levels are seen in Prolactin-producing tumors of the hypophysis, idiopathic hyperprolactinemias (symptoms: in women – alteration of the menstrual cycle, in men – impotence), hypofunction of the thyroid gland, renal insufficiency, after intake of phenothiazine derivatives, haloperidol, estrogens, oral contraceptives, histamine preparations, opiates, in hypoglycemia caused by insulin intake.

Low Prolactin levels are found after surgical resection of hypophysis, after X-ray therapy, after bromocriptine therapy, after intake of T4.

3. TEST PRINCIPLE

The determination of the prolactin is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific to prolactin murine monoclonal antibodies. Murine monoclonal antibodies to human prolactin conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage prolactin from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized prolactin
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured prolactin in the serum specimen (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of prolactin in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P206Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to prolactin; ready to use
C206Z	CAL 1	**Calibrator C1**	2 mL	1	Solution based on human serum free of prolactin, with preservative, ready to use (yellow liquid)
C206Z	CAL 2-5	**Calibrators**	0.6 mL	4	Solutions based on human serum, containing 100; 200; 1000 and 2000 mIU/L of prolactin, with preservative, ready to use (red liquids)
Q206Z	CONTROL	**Control Serum**	0.6 mL	1	Solution based on human serum, containing of known prolactin content, with preservative, ready to use (colourless liquid)
T206Z	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of murine monocnoclonal antibodies to human prolactin conjugated to the horseradish peroxidase; ready to use (red liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (1 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength or 450\620-680 nm;
– dry thermostat for 37 °C±2 °C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The Prolactin EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The Prolactin EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;

  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 2000 mIU/L, additionally dilute this sample accordingly, using (Calibrator C1). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of the blood serum (plasma) sample.

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2 If necessary, dilute the test samples as described in 9.4.

10.3 Dispense **100 µL of Conjugate Solution** to all wells.

10.4 Dispense **50 µL of Calibrators and Control Serum as well as 50 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5								
D	CAL4	CAL4	SAMP6	SAMP6								
E	CAL5	CAL5	SAMP7	SAMP7								
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.5 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.6 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the wash solution volume can be increased to 350 µL.

10.7 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.8 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.9 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the Stop Solution. Set photometer blank on CAL1.

10.10 Plot a calibration curve in linear coordinates: (x) is the prolactin concentration in the calibrators mIU/L, (y) – OD versus prolactin concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.11 Determine the corresponding concentration of prolactin in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on the results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for prolactin. Based on data obtained by XEMA, the following normal range is recommended (see below).

NOTE: values of prolactin concentrations in the tested samples that are below the LoD (5.0 mIU/L) and also exceed the value of the upper Calibrator (2000 mIU/L) should be provided in the following form : «the prolactin concentration of tested sample X is «lower than 5,00 mIU/L» or «higher than 2000 mIU/L».

Sex, age	Units, mIU/L	
	Lower limit	Upper limit
Males	60	560
Females		
Pregnancy week:		
1st trimester	-	2000
2nd trimester	-	6000
3rd trimester	-	10000
Menstrual cycle		
follicular phase	60	600
luteinic phase	120	900
ovulation	40	550

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, mIU/L	CV, %
1	11.3	7.8
2	86.45	6.4

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, mIU/L	CV, %
1	12.5	11.75
2	89.3	4.7

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, mIU/L	Concentration2, mIU/L	Concentration3, mIU/L	CV, %
1	11,2	12,03	11,87	3,76
2	86,5	89,0	95,0	4,84

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known prolactin concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 100-2000 mIU/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest prolactin concentration in the serum or plasma sample that is detected by the Prolactin EIA kit is no lower than 5 mIU/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for Prolactin EIA kit is 100mIU/L.

13.1.5 Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 2000 mIU/L.

13.1.6 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of prolactin with other analytes is shown in the table:

Analyte	Cross-reactivity, %
hGH	< 0.1
TSH	< 0.1
FSH	< 0.1
lactogen	< 0.1
LH	< 0.1

14. REFERENCES

1. Caufriez A. Menstrual disorders associated with hyperprolactinemia. HormoneRes. 1985; 22:209-14.
2. Niall, M.D. et al, «The Chemistry of Growth Hormone and the Lactogenic Hormones»; Recent Progr. Horm. Res. 29,471 (1974).
3. Frantz A.G. Physiology in medicine: prolactin. New Engl. J Med 1978; 298: 201-7.
4. Kato Y. et al. Regulation of prolactin secretion. In: The Pituitary Gland. Ed. H. Imura. New York, Raven Press. 1985; pp.261-278.
5. Thorner, M.O., Edwards, C.R.W., Hanker, J.P., Abraham, G., and Besser, G.M., «The Testes in Normal and Infertile Men», Troen, P. and Nankin, H.R. (eds.), Raven Press, New York, 351-366, (1977).
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8. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
9. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

qa@xema.com.ua

XEMA LLC
Akademika Yefremova St. 23
03179, Kyiv, Ukraine
tel.:+38 044 422-62-16
tel.:+38 044 294-69-78
E-mail: qa@xema.com.ua

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of progesterone in human serum or plasma

Progesterone EIA

Catalogue number REF **K207**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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tel.:+ 49 911 931 639 67
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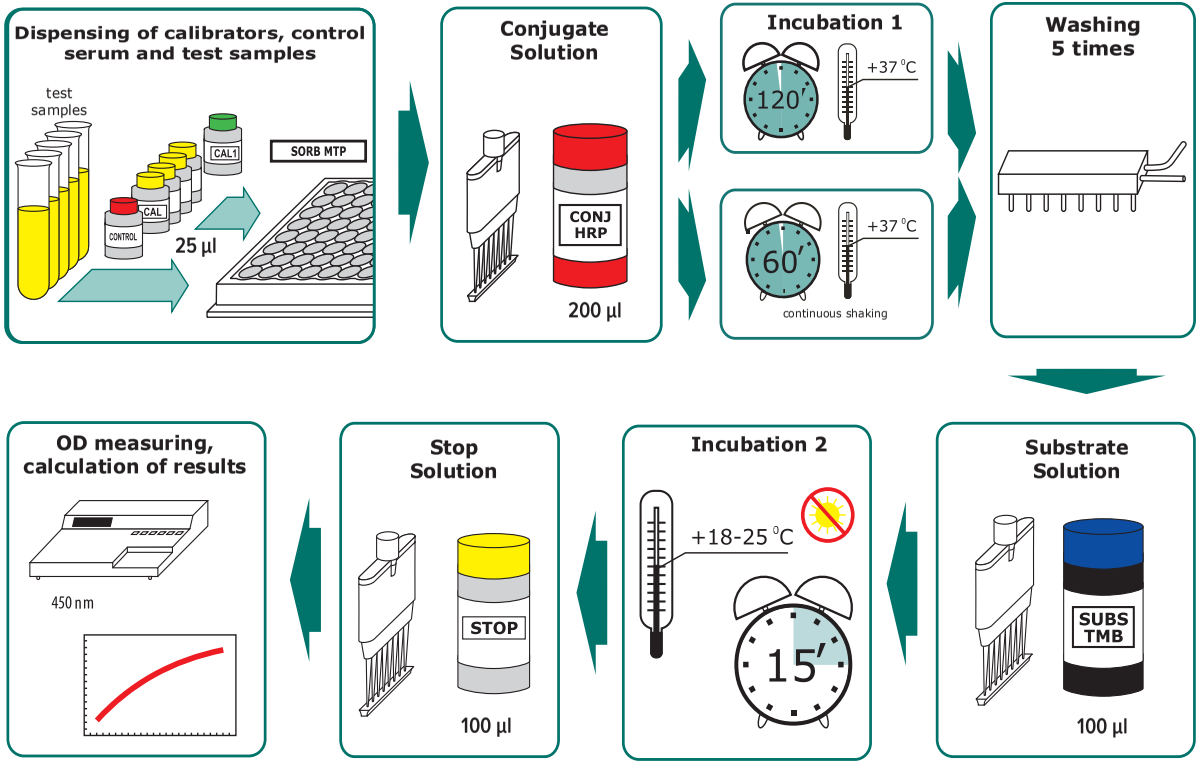
www.polmed.de

ASSAY PROCEDURE



K207

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of progesterone in human serum or plasma
Progesterone EIA

1. INTENDED USE

The Progesterone EIA kit is an enzyme immunoassay, intended for the quantitative determination of progesterone in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Progesterone is a gestagen with a MW of 314.5 Dalton. Progesterone is secreted by corpus luteum, adrenals and testis; it plays a role of a precursor for corticosteroids and androgens. Being an estrogen antagonist, Progesterony induces characteristic changes in endometrium necessary for implantation of an impregnated ovum.

During normal menstrual cycle, Progesterone level remains low until LH peak level begins to drop: only slight but statistically significant elevation of Progesterone level occurs concomitantly with LH peak followed by a decrease of Progesterone concentration. During second stage of the cycle, Progesterone and Estradiol levels increase again to complete luteinization. By the end of the cycle, Progesterone level drops again up to levels seen during follicular phase. This quick drop causes menstrual bleeding.

During pregnancy, Progesterone concentration continuously increases, and it induces proliferation and development of mammary glands and inhibits ovulation. During the first trimester, Progesterone is secreted by corpus luteum while from month 3–4 – by mitochondria of the trophoblast. Progesterone level in maternal blood increases rapidly – by week 7–8 it increases 2-fold and continues to increase by week 37–38. Decreased Progesterone levels indicate pathology of pregnancy while elevated levels suggest renal insufficiency.

Elevated Progesterone levels are found in pregnancy, tumours of adrenals or testicles, chorionepithelioma, in lipid tumours of ovaries as well as after intake of preparations of Progesterone or its analogues.

Decreased Progesterone levels are seen in galactorrhea-amenorrhea syndrome, in pregnant women at risk of premature delivery, and in persons taking some drugs such as oral contraceptives, ampicilline, ethynilestradiol.

3. TEST PRINCIPLE

The determination of the progesterone is based on the competition principle of the enzyme immunoassay. On the inner surface of the microplate wells are immobilized specific to progesterone murine monoclonal antibodies. Progesterone conjugated to the horseradish peroxidase is used as enzyme conjugate.

The analysis procedure includes two stages of incubation:

- during the first stage progesterone from the specimen competes with the conjugated progesterone for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed.
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is inversely related to the quantity of the measured progesterone in the serum specimen (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of progesterone in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P207Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to progesterone; ready to use
C207Z	CAL 1	**Calibrator C1**	0.5 mL	1	Solution based on human plasma, free of progesterone, with preservative, ready to use (yellow liquid)
C207Z	CAL 2-7	**Calibrators**	0.5 mL	6	Solutions based on human plasma, containing 1; 3; 10; 30; 100 and 300 nmol/L of progesterone, with preservative, ready to use (magenta liquids)
Q207Z	CONTROL	**Control serum**	0.5 mL	1	Solution based on human plasma, containing of known progesterone content, with preservative, ready to use (colourless liquid)
T207Z	CONJ HRP	**Conjugate Solution**	22 mL	1	Solution of progesterone conjugated to the horseradish peroxidase; ready to use (red liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (1 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength;
– dry thermostat for +37 °C±1°C or thermostat shaker maintaining a speed of 600 rpm and temperature of +37°C ±1°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The Progesterone EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The Progesterone EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- diluted washing solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

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Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

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9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

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Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

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Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 16 wells for Calibrators and Control Serum (2 wells for each calibrator (CAL 1-7) and 2 wells for control serum (Q)).

10.2 Dispense **25 µL of Calibrators and Control Serum as well as 25 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

Note: during performing several independent series of tests, Calibrators, and Control Sample should be used each time

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP1	SAMP1	SAMP9	SAMP9						
B	CAL2	CAL2	SAMP2	SAMP2	SAMP10	SAMP10						
C	CAL3	CAL3	SAMP3	SAMP3	SAMP11	SAMP11						
D	CAL4	CAL4	SAMP4	SAMP4	SAMP12	SAMP12						
E	CAL5	CAL5	SAMP5	SAMP5								
F	CAL6	CAL6	SAMP6	SAMP6								
G	CAL7	CAL7	SAMP7	SAMP7								
H	Q	Q	SAMP8	SAMP8								

10.3 Add **200 µL of the Conjugate Solution** to all wells.

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **120 minutes at +37°C**. Incubation for 60 minutes at +37°C with continuous shaking 600 rpm is allowed.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6 Add **100 µL of Substrate Solution** to all wells. The introduction of the substrate solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.7 Add **100 µL of Stop Solution** to all wells in the same order as the substrate solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.8 Read the optical density (OD) of the wells at 450nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.9 Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the progesterone concentration in the calibrators nmol/L, (y) – OD versus progesterone concentration (OD 450 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.10 Determine the corresponding concentration of progesterone in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 1.2, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

12.1. Therapeutical consequences should not be based on the results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for Progesterone. Based on data obtained by XEMA, the following normal range is recommended (see below).

NOTE: values of progesterone concentrations in the tested samples that are below the LoD (0.25 nmol/L) and also exceed the value of the upper calibrator (300 nmol/L) should be provided in the following form : «the progesterone concentration of tested sample X is «lower than 0.25 nmol/L» or «higher than 300 nmol/L».

12.2. The calibrators concentration values of the Progesterone EIA kit are expressed in nmol/L. To calculate concentrations in ng/ml, the received concentration value in nmol/L shall be multiplied by 0.318.

1 nmol/L = 0.318 ng/mL

Sex, age	Units, nmol/L		Units alternative, ng/mL	
	Lower limit	Upper limit	Lower limit	Upper limit
Males	-	4.0	-	1.27
12-17 yrs	0.3	4.3	0.1	1.37
Females				
12-17 yrs	0.3	41	0.1	13
post menopausal	-	2.3	-	0.73
Pregnancy				
1st trimester	36	240	11.4	76.3
2nd trimester	60	240	19.1	76.3
3rd trimester	156	722	49.6	229.6
Menstrual cycle				
follicular phase	0.6	4.6	0.19	1.46
luteinic phase	7.5	80	2.39	25.4
ovulation	11	80	3.5	25.4

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, nmol/L	CV, %
1	13.56	6.12
2	42.71	3.15

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, nmol/L	CV, %
1	34.25	5.02
2	124.03	4.87

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, nmol/L	Concentration2, nmol/L	Concentration3, nmol/L	CV, %
1	27.87	28.33	26.81	8.9
2	65.43	67.98	66.34	5.6

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known progesterone concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 1–300 nmol/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest progesterone concentration in the serum or plasma sample that is detected by the Progesterone EIA kit is no lower than 0.25 nmol/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for Progesterone EIA kit is 0.75 nmol/L.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of progesterone with other analytes is shown in the table:

Analyte	Cross-reactivity, %
17-Hydroxyprogesterone	1.0
11-Hydroxyprogesterone	25
Corticosterone	0.01
Pregnenolone	0.9
Deoxycorticosterone	0.3
Deoxycortisol	0.03
Cortisole	0.002

14. REFERENCES

1. Christian De Geyter, Maria De Geyter, Peter R. Huber, Eberhard Nieschlag, and Wolfgang Holzgreve – Progesterone serum levels during the follicular phase of the menstrual cycle originate from the crosstalk between the ovaries and the adrenal cortex. Hum. Reprod., Apr 2002; 17: 933 – 939.
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3. B. Gellersen, M. S. Fernandes, and J. J. Brosens – Non-genomic progesterone actions in female reproduction. Hum. Reprod. Update, Jan 2009; 15: 119 – 138.
4. J. Dinny Graham and Christine L. Clarke – Physiological Action of Progesterone in Target Tissues. Endocr. Rev., Aug 1997; 18: 502 – 519.
5. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
6. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
7. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of estradiol in human serum or plasma

Estradiol EIA

Catalogue number REF **K208**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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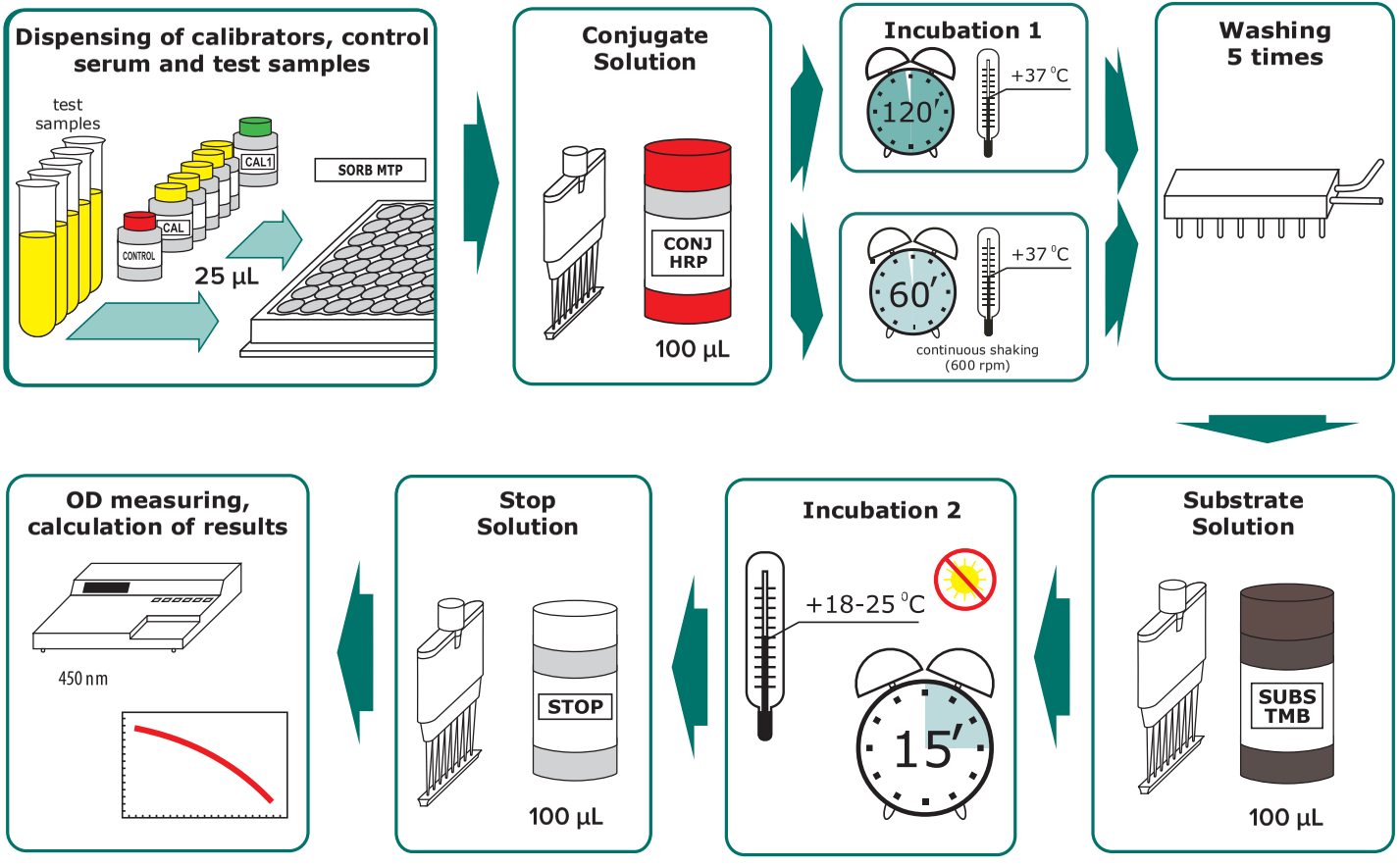
ASSAY PROCEDURE



During performing several independent series of tests, Calibrators and Control Serum should be used **each time**.

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of estradiol in human serum or plasma

Estradiol EIA

1. INTENDED USE

The Estradiol EIA kit is an enzyme immunoassay, intended for the quantitative determination of estradiol in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Estradiol (E2) is a steroid hormone with a molecular weight of 272.4 Da. It is the most active estrogen in the human body. In men, it is believed that small amounts of estradiol are produced in the adrenal cortex and testes. In the female body, it is produced in the ovaries, follicular lining and granulosa cells. The physiological role of E2 is to shape the specific functions of the female body. The secretion of E2 is regulated by the interaction of hormones from the hypothalamus, pituitary and ovaries with the participation of liberins, gonadotropins, prolactin and sex steroids. Estradiol levels remain low at the beginning and middle of the follicular phase of a normal menstrual cycle. 3-5 days before the peak of luteinising hormone (LH), E2 levels begin to rise and reach a maximum value approximately 12 hours before the peak of LH. After a sharp drop to minimum values (48 hours after the peak of LH), E2 levels begin to rise again. The maximum concentration of the hormone in the luteal phase is observed on the 9th day after ovulation, and by the end of the cycle it decreases again as the corpus luteum atresia occurs. During pregnancy, determining the level of estradiol in the blood allows you to monitor the state of the fetoplacental system. The content of E2 in the mother's blood at the beginning of pregnancy corresponds to its content in non-pregnant women during ovulation. A sharp increase in its level is observed at 9-10 weeks (12 times), then gradually increases until the end of pregnancy. A decrease in the concentration of estradiol in a dynamic study is an indicator of fetal developmental disorders. Elevated serum estradiol levels are observed in uterine bleeding during menopause, adrenal hyperplasia, estrogen-producing tumours, liver cirrhosis, feminisation in children, gonadotropins, clomiphene, and estrogens. A decrease in estradiol levels is observed in Turner's syndrome, primary and secondary hypogonadism, hermaphroditism, menopausal and postmenopausal syndromes, fetal disorders during pregnancy, and oral contraceptives.

3. TEST PRINCIPLE

The determination of the estradiol is based on the competition principle of the enzyme immunoassay. On the inner surface of the microplate wells are immobilized rabbit polyclonal antibodies to estradiol. Estradiol conjugated to the horseradish peroxidase is used as enzyme conjugate.

The analysis procedure includes two stages of incubation:

- during the first stage estradiol from the specimen competes with the conjugated estradiol for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is inversely related to the quantity of the measured estradiol in the serum specimen (plasma). The concentration of the estradiol is determined according to the calibration graph of the dependence of the optical density on the content of estradiol in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P208Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with rabbit polyclonal antibodies to estradiol, ready to use
C208Z	CAL 1	**Calibrator C1**	0.5 mL	1	Solution based on human plasma, free of estradiol, with preservative, ready to use (colourless liquid)
C208Z	CAL 2-6	**Calibrators**	0.5 mL	5	Solutions based on human plasma, containing 0.1; 0.3; 1; 3 and 20 nmol/L of estradiol, with preservative, ready to use (red liquids) *NOTE: The concentrations of estradiol in the calibration probe may slightly differ from the indicated values, the exact values are indicated on the labels of the components*
Q208Z	CONTROL	**Control serum**	0.5 mL	1	Solution based on human serum, containing of known estradiol content, with preservative, ready to use (colourless liquid)
T208Z	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of estradiol conjugated to the horseradish peroxidase, ready to use (red liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution, ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid, ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (1 pcs.).					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength;
– dry thermostat for +37 °C±1°C or thermostat shaker maintaining a speed of 600 rpm and temperature of +37°C ±1°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The Estradiol EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The Estradiol EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.
- diluted washing solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

10. ASSAY PROCEDURE

10.1. Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 14 wells for Calibrators and Control Serum (2 wells for each calibrator (CAL 1-6) and 2 wells for control serum (Q)).

10.2. Dispense **25 µL of Calibrators and Control Serum as well as 25 µL of test serum/ plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators and Control Sample should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5								
E	CAL5	CAL5	SAMP6	SAMP6								
F	CAL6	CAL6	SAMP7	SAMP7								
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.3. Add **100 µL of the Conjugate Solution** to all wells.

10.4. Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **120 minutes at +37°C**. Incubation for 60 minutes at +37°C with continuous shaking 600 rpm is allowed.

10.5. At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well **5 times** using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5 µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6. Add **100 µL of Substrate Solution** to all wells. The introduction of the substrate solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.7. Add **100 µL of Stop Solution** to all wells in the same order as the substrate solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.8. Read the optical density (OD) of the wells at 450 nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.9. Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the testosterone concentration in the calibrators nmol/L, (y) – OD versus testosterone concentration (OD 450 nm). Manual or computerized data reduction is applicable at this stage. For the algorithm calculation (approximation) of the calibration curve, using the interval (segment-linear, point-to-point) method is recommended. Adjust the concentration of CAL1 to an infinitesimally small value, for example, 0.001 nmol/L.

10.10. Determine the corresponding concentration of estradiol in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 1.2, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

12.1. Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for testosterone. Based on data obtained by XEMA LLC, the following normal range is recommended (see below).

NOTE: values of estradiol concentrations in the tested samples that are below the LoD (0.025 nmol/L) and also exceed the value of the upper calibrator (20 nmol/L) should be provided in the following form: «the estradiol concentration of tested sample X is «lower than 0.025 nmol/L» or «higher than 20 nmol/L».

12.2. The calibrators concentration values of the Estradiol EIA kit are expressed in nmol/L. To calculate concentrations in pg/mL, the received concentration value in nmol/L shall be multiplied by 272.

1 nmol/L = 272 pg/mL

Sex, age	Units, nmol/L		Units alternative, pg/mL	
	Lower limit	Upper limit	Lower limit	Upper limit
Males	0.029	0.3	7.9	81.6
Children < 11 yrs	-	0.2	-	54.4
Females				
Pregnancy week:				
1st trimester	0.1	10.5	27	2856
2nd trimester	3.0	21	816	5712
3rd trimester	6.0	80	1632	21760
Menstrual cycle:				
follicular phase	0.05	0.7	13.6	190.4
lutea phase	0.1	1.1	27.2	299.2
ovulation	0.34	1.8	92.5	489.6
menopause	-	0.23	-	62.6

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1. Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, nmol/L	CV, %
1	6.91	3.8
2	1.96	5.3

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, nmol/L	CV, %
1	6.87	4.0
2	2.02	6.2

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, nmol/L	Concentration2, nmol/L	Concentration3, nmol/L	CV, %
1	3.45	3.09	3.24	6.5
2	9.01	9.64	8.97	8.1

13.1.2. Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3. Linearity

Linearity was determined using sera samples with known estradiol concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 0.05–10nmol/L ±10%.

13.1.4. Analytical sensitivity

Limit of detection (LoD) – the lowest estradiol concentration in the serum or plasma sample that is detected by the Estradiol EIA kit is no lower than 0.025 nmol/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for Estradiol EIA kit is 0.05 nmol/L.

13.1.5. Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of estradiol with other analytes is shown in the table:

Analyte	Cross-reactivity, %
Estrone	0.2
Estriol	0.6
Cortisol	0.06
Prednisol	0.09
Corticosterone	<0.01
Progesterone	<0.01
17-OH progesterone	<0.05
Pregnenolone	<0.05
Testosterone	<0.01

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9. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
10. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81).

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
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LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of testosterone in human serum or plasma

Testosterone EIA

Catalogue number REF **K209**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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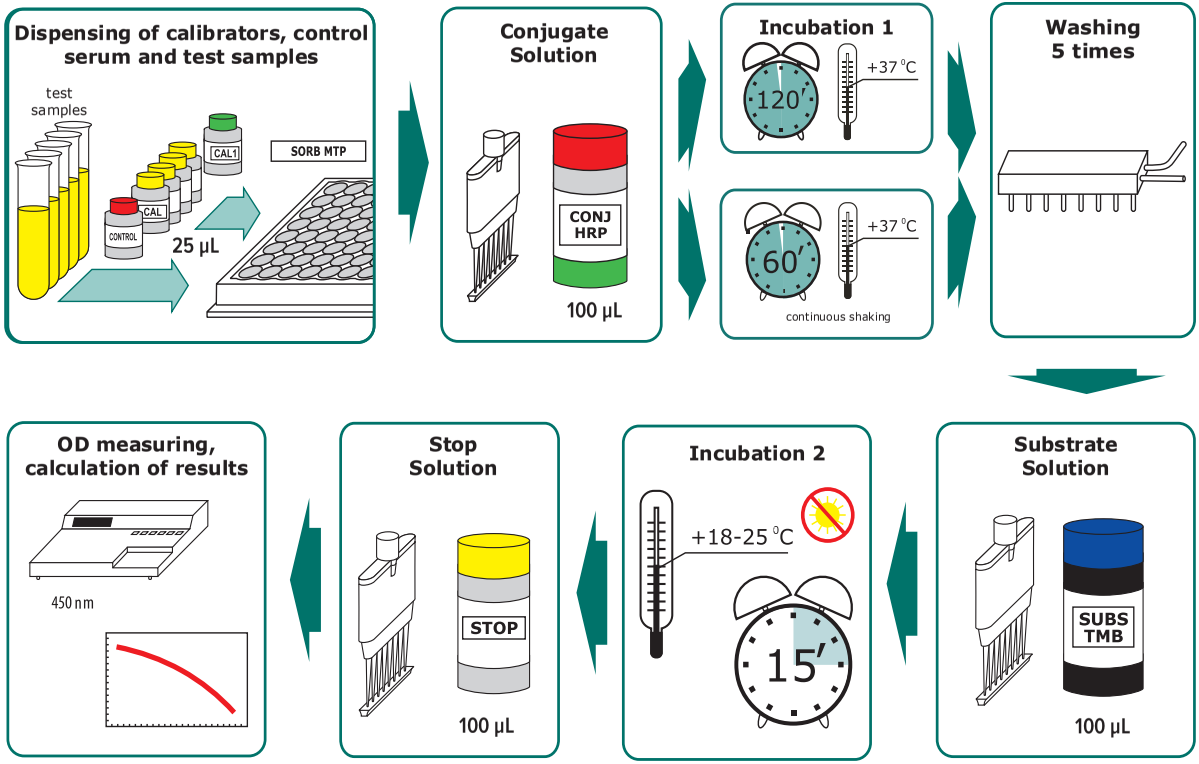
ASSAY PROCEDURE



K209

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of testosterone in human serum or plasma
Testosterone EIA

1. INTENDED USE

The Testosterone EIA kit is an enzyme immunoassay, intended for the quantitative determination of testosterone in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Testosterone is a steroid with a MW of 288.4 Dalton. The main sites of testosterone secretion are Leidig cells in interstitial tissue of testicles in men. In women testosterone is secreted in the adrenals and is controlled by luteinizing hormone. Testosterone stimulates development of male genital organs and formation of secondary sexual features.

In males, testosterone secretion undergoes circadian rhythms with maximal concentrations seen in the morning (6 am) and minimal – in the evening (8 pm). In females, testosterone secretion is regulated by menstrual cycle with maximal levels found in luteinic phase and during ovulation.

Leidig cell tumours producing high levels of serum testosterone in young boys lead to development of "little Hercules" syndrome. Elevated testosterone level in women causes the clinical signs of masculinization.

In men, decreased testosterone levels may lead to female habitus or underdevelopment of male genital organs in boys. To differentiate between primary and secondary hypogonadism, testosterone should be assayed in conjunction with LH and FSH.

3. TEST PRINCIPLE

The determination of the testosterone is based on the competition principle of the enzyme immunoassay. On the inner surface of the microplate wells are immobilized specific to testosterone murine monoclonal antibodies. Testosterone conjugated to the horseradish peroxidase is used as enzyme conjugate.

The analysis procedure includes two stages of incubation:

- during the first stage testosterone from the specimen competes with the conjugated testosterone for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed.
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is inversely related to the quantity of the measured testosterone in the serum specimen (plasma). The concentration of the testosterone is determined according to the calibration graph of the dependence of the optical density on the content of testosterone in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P209Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to testosterone; ready to use
C209Z	CAL 1	**Calibrator C1**	0.5 mL	1	Solution based on human plasma, free of testosterone, with preservative, ready to use (colourless liquid)
C209Z	CAL 2-6	**Calibrators**	0.5 mL	5	Solutions based on human plasma, containing 1; 3; 10; 30 and 100 nmol/L of testosterone, with preservative, ready to use (blue liquids)
Q209Z	CONTROL	**Control serum**	0.5 mL	1	Solution based on human serum, containing of known testosterone content, with preservative, ready to use (blue liquid)
T209Z	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of testosterone conjugated to the horseradish peroxidase; ready to use (green liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (1 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm wavelength;
- dry thermostat for +37 °C±2°C or thermostat shaker maintaining a speed of 600 rpm and temperature of +37°C ±1°C;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- vortex mixer;
- disposable gloves;
- absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The Testosterone EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The Testosterone EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;

  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.
- diluted washing solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 14 wells for Calibrators and Control Serum (2 wells for each calibrator (CAL 1-6) and 2 wells for control serum (Q)).

10.2 Dispense **25 µL of Calibrators and Control Serum as well as 25 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

Note: during performing several independent series of tests, Calibrators, and Control Sample should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5								
E	CAL5	CAL5	SAMP6	SAMP6								
F	CAL6	CAL6	SAMP7	SAMP7								
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.3 Add **100 µL of the Conjugate Solution** to all wells.

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **120 minutes at +37°C**. Incubation for 60 minutes at +37°C with continuous shaking 600 rpm is allowed.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6 Add **100 µL of Substrate Solution** to all wells. The introduction of the substrate solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.7 Add **100 µL of Stop Solution** to all wells in the same order as the substrate solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.8 Read the optical density (OD) of the wells at 450nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.9 Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the testosterone concentration in the calibrators nmol/L, (y) – OD versus testosterone concentration (OD 450 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.10 Determine the corresponding concentration of testosterone in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 1.2, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

12.1. Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for testosterone. Based on data obtained by XEMA LLC, the following normal range is recommended (see below).

NOTE: values of testosterone concentrations in the tested samples that are below the LoD (0.15nmol/L) and also exceed the value of the upper calibrator (100 nmol/L) should be provided in the following form : «the testosterone concentration of tested sample X is «lower than 0.15 nmol/L» or «higher than 100 nmol/L».

12.2. The calibrators concentration values of the Testosterone EIA kit are expressed in nmol/L. To calculate concentrations in ng/mL, the received concentration value in nmol/L shall be multiplied by 0.29.

1 nmol/L = 0,29 ng/mL

Sex, age	Units, nmol/L		Units alternative, ng/mL	
	Lower limit	Upper limit	Lower limit	Upper limit
Males				
20-39 yrs	9.0	38	2.6	11
40-55 yrs	6.9	21	2.0	6.1
>55 yrs	5.9	18.1	1.7	5.2
Females	-	4.6	-	1.3

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, nmol/L	CV, %
1	93.16	1.63
2	28.5	7.87

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, nmol/L	CV, %
1	95.34	5.25
2	28.47	2.57

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, nmol/L	Concentration2, nmol/L	Concentration3, nmol/L	CV, %
1	94.6	95.89	97.89	1.72
2	28.4	27.75	29.46	3.02

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known testosterone concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 1–100nmol/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest testosterone concentration in the serum or plasma sample that is detected by the Testosterone EIA kit is no lower than 0.15 nmol/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for Testosterone EIA kit is 1.0 nmol/L.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of testosterone with other analytes is shown in the table:

Analyte	Cross-reactivity, %
5-alpha-dehydrotestosterone	16
Androstendiol	1
Androstendione	0.4
Androsterone	<0.1
Dehydroepiandrosterone	<0.1
Progesterone	<0.1
Estradiol, Estriol	<0.01
Cortisol, Pregnenolone	<0.01

14. REFERENCES

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6. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
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LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of cortisol in human serum or plasma

Cortisol EIA

Catalogue number REF **K210**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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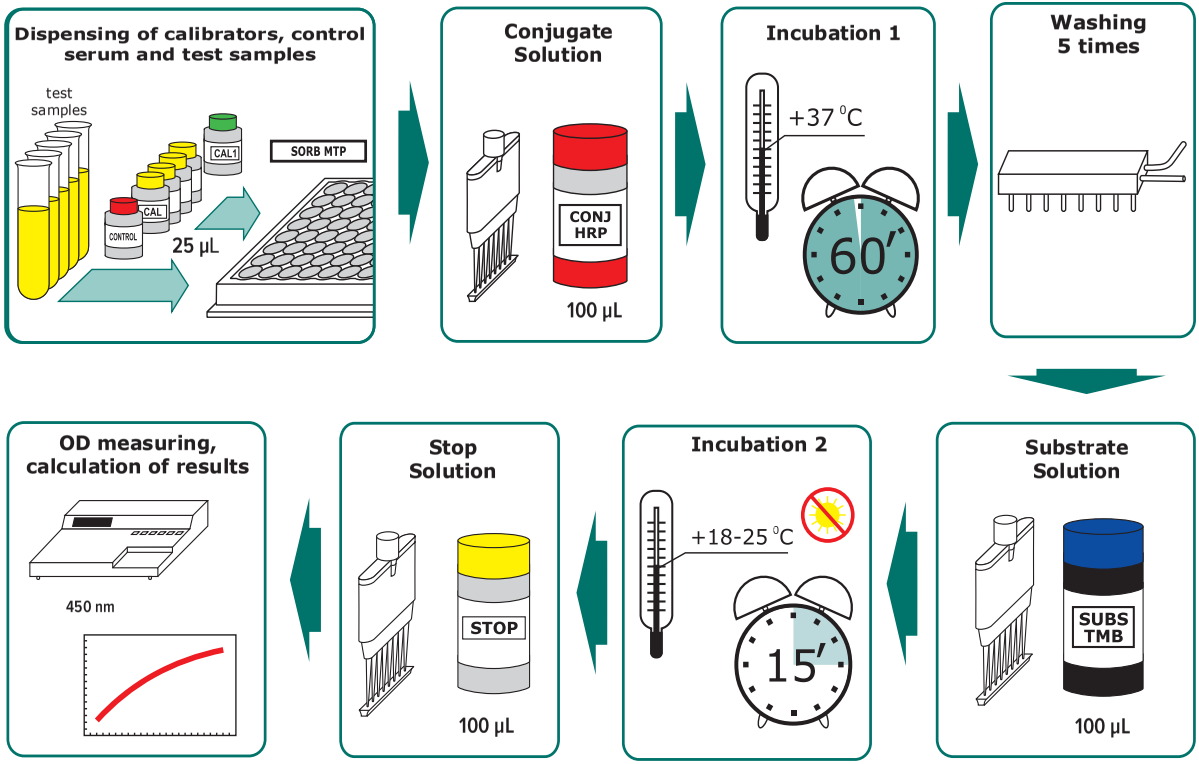
ASSAY PROCEDURE



K210

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of cortisol in human serum or plasma
Cortisol EIA

1. INTENDED USE

The Cortisol EIA kit is an enzyme immunoassay, intended for the quantitative determination of cortisol in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Cortisol is a glucocorticoid with a MW of 362.5 Dalton. Cortisol is the major hormone secreted by adrenals. In blood, cortisol is found mostly in a bound form, transcortin being the carrier. Cortisol secretion undergoes circadian rhythms with maximal (up to 700 nmol/L) concentrations seen in the morning (6–9 am) and minimal (up to 55 nmol/L) – in the midnight.

During pregnancy, Cortisol blood level is continuously increasing by up to 5-fold of initial concentration before delivery, its circadian rhythm being altered. Cortisol plays an important role in development of alveolar epithelium and surfactant secretion, this being of major importance for the first inhale of a newborn.

Elevated Cortisol concentrations in blood are found in secreting tumours of adrenals, in virilizing hyperplasia of adrenals, in Cushing syndrome, in ACTH-producing tumours, during surgical stress, in cardiac insufficiency, diabetes, burns, pains, during pregnancy, during estrogen therapy, etc. Cortisol blood level may be increased by intake of ACTH, Cortisol, alcohol, nicotine, oral contraceptives.

Decreased Cortisol levels are found in Addison syndrome, adrenogenital syndrome, hypopituitarism. Some drugs may decrease Cortisol level in blood, such as: L-DOPA, dexamethasone, etc. Decreased Cortisol level during pregnancy may indicate anencephaly of the fetus.

3. TEST PRINCIPLE

The determination of cortisol is based on the competition principle of the enzyme immunoassay. On the inner surface of the microplate wells are immobilized specific to cortisol murine monoclonal antibodies. Cortisol conjugated to the horseradish peroxidase is used as enzyme conjugate.

The analysis procedure includes two stages of incubation:

- during the first stage cortisol from the specimen competes with the conjugated cortisol for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed.
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is inversely related to the quantity of the measured cortisol in the serum specimen (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of cortisol in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P210Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to cortisol; ready to use
C210Z	CAL 1	**Calibrator C1**	0.5 mL	1	Solution based on human plasma, free of cortisol, with preservative, ready to use (yellow liquid)
C210Z	CAL 2-6	**Calibrators**	0.5 mL	5	Solutions based on human plasma, containing 40; 80; 200; 600 and 2000 nmol/L of cortisol, with preservative, ready to use (blue liquids)
Q210Z	CONTROL	**Control serum**	0.5 mL	1	Solution based on human plasma, containing of known cortisol content, with preservative, ready to use (colourless liquid)
T210Z	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of cortisol conjugated to the horseradish peroxidase; ready to use (red liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (1 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm wavelength;
- dry thermostat for +37 °C±1°C or thermostat shaker maintaining a speed of 600 rpm and temperature of +37°C ±1°C;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- vortex mixer;
- disposable gloves;
- absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The Cortisol EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The Cortisol EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;

  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 14 wells for Calibrators and Control Serum (2 wells for each calibrator (CAL 1-6) and 2 wells for control serum (Q)).

10.2 Dispense **25 µL of Calibrators and Control Serum as well as 25 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

Note: during performing several independent series of tests, Calibrators, and Control Sample should be used each time

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5								
E	CAL5	CAL5	SAMP6	SAMP6								
F	CAL6	CAL6	SAMP7	SAMP7								
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.3 Add **100 µL of the Conjugate Solution** to all wells.

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**. Incubation for 30 minutes at +37°C with continuous shaking 600 rpm is allowed.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6 Add **100 µL of Substrate Solution** to all wells. The introduction of the substrate solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.7 Add **100 µL of Stop Solution** to all wells in the same order as the substrate solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.8 Read the optical density (OD) of the wells at 450nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.9 Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the cortisol concentration in the calibrators nmol/L, (y) – OD versus cortisol concentration (OD 450 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve. Adjust the concentration of CAL1 to an infinitesimally small value, for example, 0.001 nmol/L.

10.10 Determine the corresponding concentration of cortisol in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 1.2, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

12.1. Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for Cortisol. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

NOTE: values of cortisol concentrations in the tested samples that are below the LoD (6.0 nmol/L) and also exceed the value of the upper calibrator (2000 nmol/L) should be provided in the following form : «the cortisol concentration of tested sample X is «lower than 6.0 nmol/L» or «higher than 2000 nmol/L».

Sex, age	Units, nmol/L	
	Lower limit	Upper limit
Healthy donors	140	600

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, nmol/L	CV, %
1	110	5.5
2	264.65	4.98

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, nmol/L	CV, %
1	114.3	9.3
2	256	11.2

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, nmol/L	Concentration2, nmol/L	Concentration3, nmol/L	CV, %
1	111.5	120	116.3	3.68
2	260.3	265.5	263	0.99

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known cortisol concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 40–2000 nmol/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest cortisol concentration in the serum or plasma sample that is detected by the Cortisol EIA kit is no lower than 6.0 nmol/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for Cortisol EIA kit is 40 nmol/L.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of cortisol with other analytes is shown in the table:

Analyte	Cross-reactivity, %
11-Deoxycortisol	0.9
Prednisolone	5.6
Corticosterone	0.6
11-Deoxycorticosterone	<0.1
Progesterone	<0.1
17-Hydroxyprogesterone	<0.1

14. REFERENCES

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9. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
10. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

qa@xema.com.ua

XEMA LLC
Akademika Yefremova St. 23
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Instruction for use
A solid-phase enzyme immunoassay kit
for the quantitative determination of
triiodothyronine in human serum or plasma

T3 EIA

Catalogue number REF **K211**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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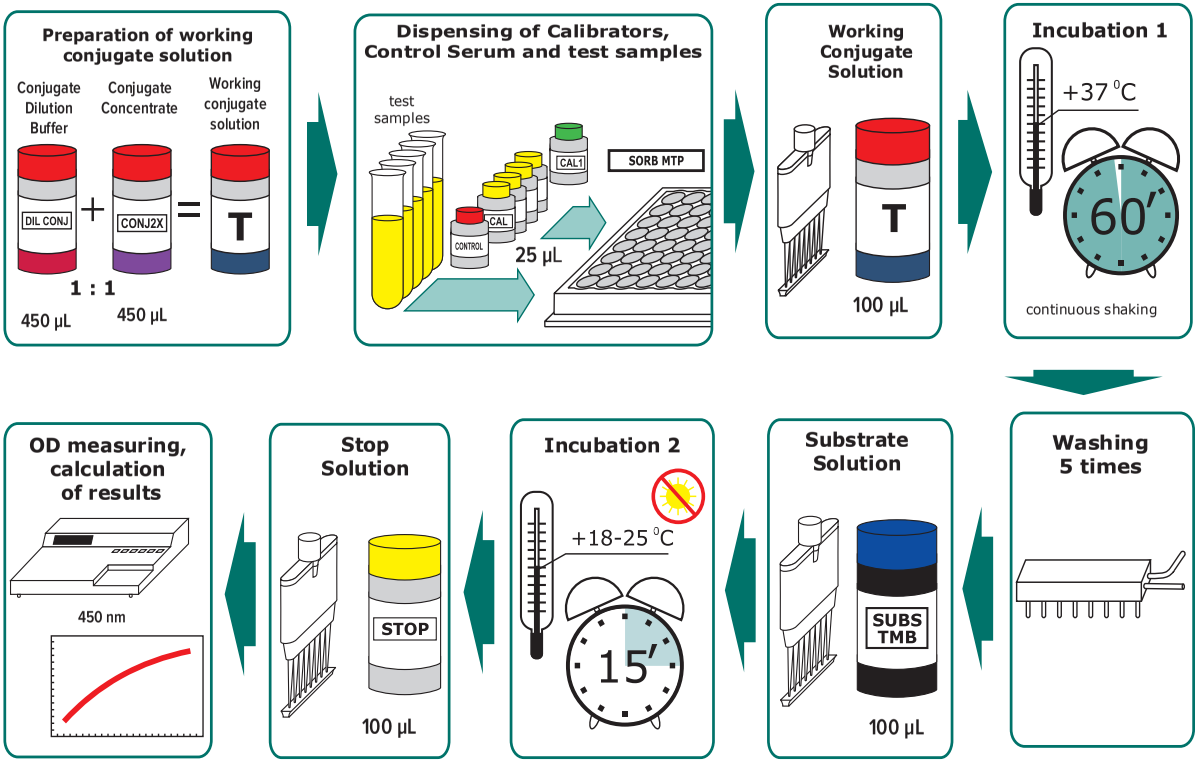
ASSAY PROCEDURE



K211

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of triiodothyronine in human serum or plasma
T3 EIA

1. INTENDED USE

The T3 EIA kit is an enzyme immunoassay, intended for the quantitative determination of triiodothyronine in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Triiodothyronine (T3) is a hormone with a molecular weight of 651 Da, 58% of which is iodine. Thyroid hormones thyroxin (T4) and 3,5,3′-triiodothyronine (T3) exert regulatory influences on growth, differentiation, cellular metabolism and development of skeletal and organ systems. T4 and T3 in blood are found both in free and bound form – mostly, they are bound to thyroxin binding globulin (TBG). Only free forms of T3 and T4 exert hormonal activity also their percentage is very low – 0.3% for T3 and 0.03% for T4.

The concentration of T3 is much less than that of T4 but its metabolic activity is about 3 times greater. About 80% of T3 is produced in peripheral tissues by deiodination of T4, and only 20% is secreted by thyroid gland. That is why in hypothyroid patients T3 level may for a long time remain on the lower limit of the normal range, because its loss may be compensated by enhanced conversion of T4 into T3.

Determination of T3 level is most useful in T3-hyperthyroidism because 5-10% of such patients do not show significant changes in T4 level while concentration of T3 is highly elevated. Elevated T3 levels are seen in early thyroid hypofunction, after intake of estrogens, oral contraceptives, heroin, methadone, during pregnancy.

Decreased concentrations of T3 are found in initial stage of hyperthyroidism, acute and subacute thyroiditis, after intake of androgens, dexamethasone, salycilates. Decreased concentrations of T3 are found in initial stage of hyperthyroidism, acute and subacute thyroiditis, after intake of androgens, dexamethasone, salycilates.

3. TEST PRINCIPLE

The determination of triiodothyronine is based on the competition principle of the enzyme immunoassay. On the inner surface of the microplate wells are immobilized specific rabbit polyclonal to T3 antibodies. T3 conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage T3 from the specimen competes with the conjugated T3 for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed.
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3′,5,5′-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is inversely related to the quantity of the measured T3 in the serum specimen (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of T3 in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P211Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with rabbit polyclonal antibodies to T3, ready to use;
C211Z	CAL 1	**Calibrator C1**	0.5 mL	1	Solution based on tris buffer (pH 7.2-7.4), free of T3, with preservative, ready to use (yellow liquid)
C211Z	CAL 2-5	**Calibrators**	0.5 mL	4	Solutions based on tris buffer (pH 7.2-7.4), containing 0,75; 1,5; 7,5 and 15 nmol/L of T3, with preservative, ready to use (blue liquids)
Q211Z	CONTROL	**Control serum**	0.5 mL	1	Solution based on human plasma, containing of known T3 content, with preservative, ready to use (colourless liquid)
T211XZ	CONJ 2X	**Conjugate Concentrate**	7 mL	1	Solution of T3 conjugated to the horseradish peroxidase; 2x concentrate (purple liquid)
ST211Z	DIL CONJ	**Conjugate Dilution Buffer**	7 mL	1	Buffer solution with detergent ready to use (red liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 ml (мл)	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 ml (мл)	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 ml (мл)	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm wavelength;
- shaker maintaining a speed of 500 rpm for +37 °C±2°C;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- vortex mixer;
- disposable gloves;
- absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The T3 EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The T3 EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Concentrate, Conjugate Dilution Buffer, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

9.4. Working conjugate solution preparation

Prepare a working conjugate solution by 2 dilutions of Conjugate Concentrate in Conjugate Dilution Buffer (eg, 450 µL of concentrate + 450 µL of Conjugate Dilution Buffer). In the case of partial use of the kit, take the necessary amount of Conjugate Concentrate and dilute it 2 times with Conjugate Dilution Buffer, since the working conjugate solution in a diluted form is not stored for a long time.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550
Volume of Conjugate Concentrate, mL	0.45	0.9	1.35	1.8	2.25	2.7	3.15	3.6	4.05	4.5	4.95	5.4
Volume of Conjugate Dilution Buffer, mL	0.45	0.9	1.35	1.8	2.25	2.7	3.15	3.6	4.05	4.5	4.95	5.4

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each calibrator (CAL 1-5) and 2 wells for control serum (Q)).

10.2 Prepare Working conjugate solution as described in 9.4.

10.3 Dispense **25 µL of Calibrators and Control Serum as well as 25 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

Note: during performing several independent series of tests, Calibrators, and Control Sample should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5								
D	CAL4	CAL4	SAMP6	SAMP6								
E	CAL5	CAL5	SAMP7	SAMP7								
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.4 Dispense **100 µL of Working conjugate solution** to all wells.

10.5 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C with continuous shaking 500 rpm**.

10.6 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.7 Add **100 µL of Substrate Solution** to all wells. The introduction of the substrate solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.8 Add **100 µL of Stop Solution** to all wells in the same order as the substrate solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.9 Read the optical density (OD) of the wells at 450nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.10 Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the T3 concentration in the calibrators nmol/L, (y) – OD versus T3 concentration (OD 450 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve. Adjust the concentration of CAL1 to an infinitesimally small value, for example, 0.001 nmol/L.

10.11 Determine the corresponding concentration of T3 in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 1.2, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for T3. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

NOTE: values of T3 concentrations in the tested samples that are below the LoD (0.2 nmol/L) and also exceed the value of the upper calibrator (15 nmol/L) should be provided in the following form: «the T3 concentration of tested sample X is «lower than 0.2 nmol/L» or «higher than 15 nmol/L».

The concentration values of the T3 EIA kit calibrators are expressed in nmol/L. To convert the concentration in ng/mL it is necessary to multiply by 0.65 the obtained concentration value in nmol/L.

1 nmol/L = 0.65 ng/mL

Sex, age	Units, nmol/L		Units alternative, ng/mL	
	Lower limit	Upper limit	Lower limit	Upper limit
Healthy donors	1.2	3.2	0.8	2.1

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, nmol/L	CV, %
1	2.32	9.16
2	1.45	9.66

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, nmol/L	CV, %
1	1.38	9.89
2	1.75	8.41

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, nmol/L	Concentration2, nmol/L	Concentration3, nmol/L	CV, %
1	2.12	2.02	2.27	13.9
2	1.56	1.44	1.81	15.6

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known T3 concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 0.75 –15 nmol/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest T3 concentration in the serum or plasma sample that is detected by the T3 EIA kit is no lower than 0.2 nmol/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for T3 EIA kit is 0.55 nmol/L.

3.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of T3 with other analytes is shown in the table:

Analyte	Cross-reactivity, %
L-Thyroxin	0.01
D-Thyroxin	0.04

14. REFERENCES

1. Physiology of thyroid hormones. IN: Division of Drugs and Toxicology, American Medical Association: Drug Evaluations Annual 1995. Amer Med Assn, Chicago, 1995, ch 47, pp 1039-1040.
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4. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
5. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of thyroxin in human serum or plasma

T4 EIA

Catalogue number REF **K212**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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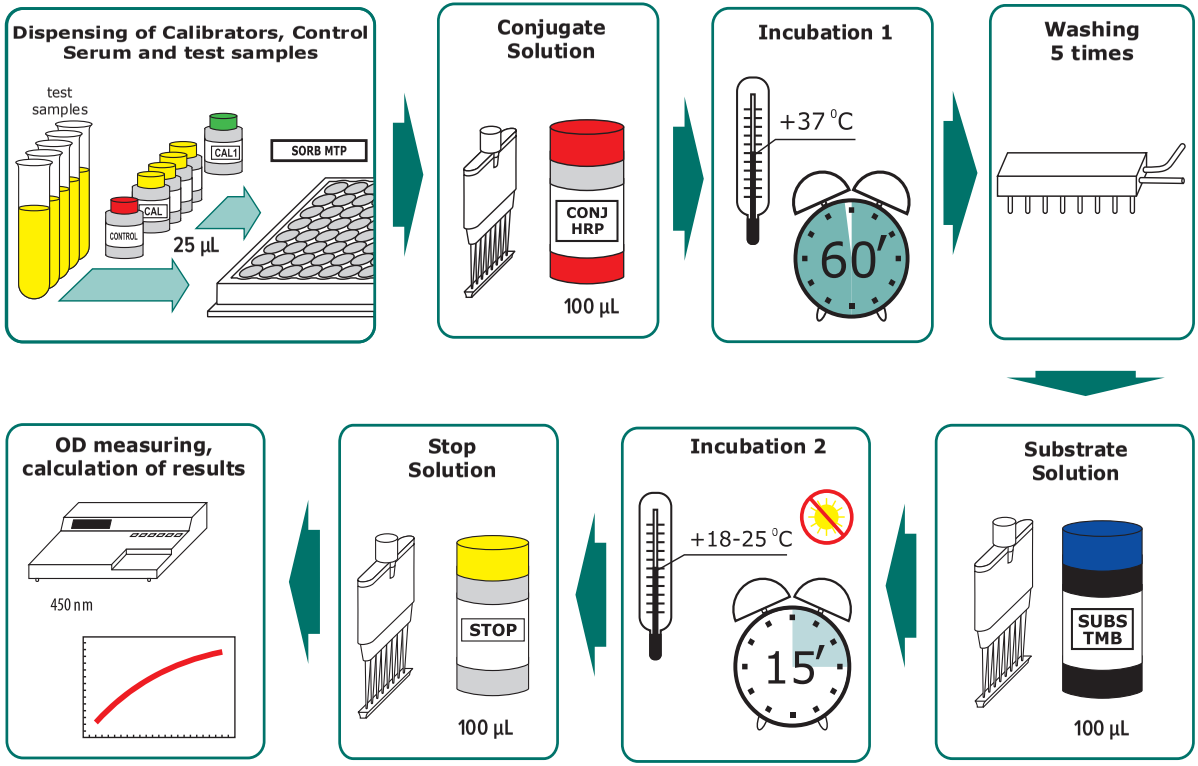
ASSAY PROCEDURE



K212

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of thyroxin in human serum or plasma
T4 EIA

1. INTENDED USE

The T4 EIA kit is an enzyme immunoassay, intended for the quantitative determination of thyroxin in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Thyroxine (T4) and triiodothyronine (T3) are hormones that are produced by the thyroid gland and circulate in the blood both free and bound - mainly with thyroxine-binding globulin (TBG). Only free T3 and T4 are characterized by Hormonal activity, but their share is very small: 0.03% of the total content for T4 and 0.3% - for T3. Concentration of T4 in serum blood is the most accepted indicator of thyroid gland function, which allows you to clearly distinguish between hyper-, hypo- and euthyroidism.

Increase of total T4 concentration is observed with hyperthyroidism, with pituitary tumors, with conditions with elevated TSH levels (pregnancy, acute or chronic active hepatitis, estrogen-secreting tumors or estrogen intake, genetically conditional increase), while taking oral contraceptives, heroin, methadone, thyroid drugs, TSH, thyroliberin.

Decrease of total T4 concentration is observed in hypothyroidism, panhypopituitarism, states of low levels of TSH (acromegaly, nephrotic syndrome, hypoproteinemia, chronic liver disease, androgen-secreting tumors, or androgens, genetically determined decrease), hemolysis, exercise, when taking amino salicylic and acetylsalicylic acids, glucocorticoids, sulfonamides, cholestyramine, reserpine, potassium iodide, triiodothyronine.

3. TEST PRINCIPLE

Determination of the thyroxine is based on competition principle of the enzyme immunoassay. Microwells plate is coated with specific murine monoclonal to thyroxine antibodies. Thyroxine conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage thyroxine from the specimen competes with the conjugated thyroxine for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed.
- during the second stage, the complexes formed due the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. Optical density in the microwell is inversely related to the quantity of the measured thyroxine in the specimen of the serum (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of thyroxine in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P212Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to T4; ready to use
C212Z	CAL 1	**Calibrator C1**	0.5 mL	1	Solution based on human plasma, free of thyroxin, with preservative, ready to use (yellow liquid)
C212Z	CAL 2-5	**Calibrators**	0.5 mL	4	Solutions based on human plasma, containing 32; 64; 160 and 320 nmol/L of thyroxin, with preservative, ready to use (red liquids)
Q212Z	CONTROL	**Control Serum**	0.5 mL	1	Solution based on human plasma, containing of known thyroxin content, with preservative, ready to use (colourless liquid)
T212XZ	CONJ	**Conjugate Solution**	14 mL	1	Solution of thyroxin conjugated to the horseradish peroxidase; ready to use (red liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength;
– dry thermostat for +37°C±2°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The T4 EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The T4 EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;

  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed
- diluted washing solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each calibrator (CAL 1-5) and 2 wells for control serum (Q)).

10.2 Dispense **25 µL of Calibrators and Control Serum as well as 25 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

Note: during performing several independent series of tests, Calibrators, and Control Sample should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5								
D	CAL4	CAL4	SAMP6	SAMP6								
E	CAL5	CAL5	SAMP7	SAMP7								
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.3 Add **100 µL of the Conjugate Solution** to all wells.

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6 Add **100 µL of Substrate Solution** to all wells. The introduction of the substrate solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.7 Add **100 µL of Stop Solution** to all wells in the same order as the substrate solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.8 Read the optical density (OD) of the wells at 450nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.9 Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the T4 concentration in the calibrators nmol/L, (y) – OD versus T4 concentration (OD 450 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve. Adjust the concentration of CAL1 to an infinitesimally small value, for example, 0.001 nmol/L.

10.10 Determine the corresponding concentration of T4 in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 1.2, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

12.1. Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for T4. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying

NOTE: values of T4 concentrations in the tested samples that are below the LoD (3.0 nmol/L) and also exceed the value of the upper calibrator (320 nmol/L) should be provided in the following form : «the T4 concentration of tested sample X is «lower than 3.0 nmol/L» or «higher than 320 nmol/L».

12.2. The calibrators concentration values of the T4 EIA kit are expressed in nmol/L. To calculate concentrations in µg/dl, the received concentration value in nmol/L shall be multiplied by 0.0775.

1 nmol/L = 0.0775 µg/dl

Sex, age	Units, nmol/L		Units alternative, µg/dl	
	Lower limit	Upper limit	Lower limit	Upper limit
Healthy donors	60	160	4.7	12.4
Males				
>61 yrs	60	129	4.7	10.0
Females				
>61 yrs	70	135	5.4	10.5
Children				
1-5 yrs	90	190	7.0	14.7
6-10 yrs	83	170	6.4	13.2
>10 yrs	60	160	4.7	12.4

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

3.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, nmol/L	CV, %
1	17.5	4.36
2	110.7	3.67

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, nmol/L	CV, %
1	16.4	1.17
2	111.1	5.43

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, nmol/L	Concentration2, nmol/L	Concentration3, nmol/L	CV, %
1	14.59	13.67	15.39	5.92
2	116.23	114.53	120.13	2.45

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known T4 concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 0.75–15 nmol/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest T4 concentration in the serum or plasma sample that is detected by the T4 EIA kit is no lower than 3 nmol/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for T4 EIA kit is 32 nmol/L.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of T4 with other analytes is shown in the table:

Analyte	Cross-reactivity, %
T3	0.5
D-Thyroxin	30

14. REFERENCES

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7. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
8. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

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LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

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LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

qa@xema.com.ua

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Instruction for use
A solid-phase enzyme immunoassay kit
for the quantitative determination of
free triiodothyronine in human serum or plasma

fT3 EIA

Catalogue number REF **K213**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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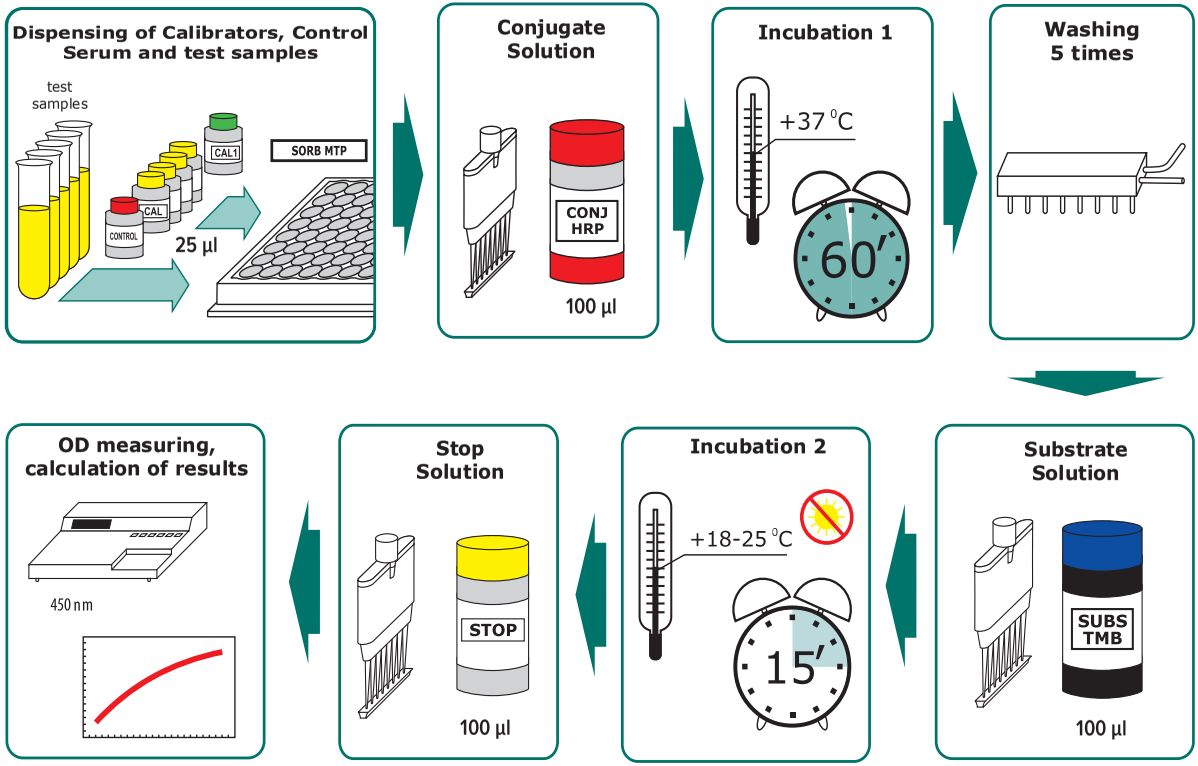
ASSAY PROCEDURE



K213

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of free triiodothyronine in human serum or plasma
fT3 EIA

1. INTENDED USE

The fT3 EIA kit is an enzyme immunoassay, intended for the quantitative determination of free triiodothyronine in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Thyroxine (T4) and triiodothyronine (T3) are hormones that are produced by the thyroid gland and circulate in the blood both free and bound - mainly with thyroxine-binding globulin (TBG). Only free T3 and T4 are characterized by Hormonal activity, but their share is very small: 0.03% of the total content for T4 and 0.3% - for T3.

The concentration of T3 is much less than that of T4 but its metabolic activity is about 3 times greater. About 80% of T3 is produced in peripheral tissues by deiodination of T4, and only a small amount of it is secreted by thyroid gland. That is why in hypothyroid patients T3 level may for a long time remain on the lower limit of the normal range, because its loss may be compensated by enhanced conversion of T4 into T3.

The determination of total and free T3 concentration is carried out at the initial stage of hyperthyroidism, in case of recurrence of hyperthyroidism, in the differential diagnosis of hyperthyroidism, in case of a symptomatic increase of the T3 level, in case of acute hyperthyroidism after suppressive therapy with L-thyroxine.

3. TEST PRINCIPLE

Determination of the fT3 is based on competition principle of the enzyme immunoassay. Microwells plate is coated with specific rabbit polyclonal to T3 antibodies. fT3 conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage fT3 from the specimen competes with the conjugated fT3 for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed.
- during the second stage, the complexes formed due the reaction with the chromogen 3,3′,5,5′-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. Optical density in the microwell is inversely related to the quantity of the measured fT3 in the specimen of the serum (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of fT3 in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P213Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with rabbit polyclonal antibodies to T3; ready to use
C213Z	CAL 1	**Calibrator C1**	0.5 mL	1	Solution based on human plasma, free of fT3, with preservative, ready to use (yellow liquid)
C213Z	CAL 2-6	**Calibrators**	0.5 mL	5	Solutions based on human plasma, containin 2,5; 5; 10; 20 and 40 pmol/L of fT3, with preservative, ready to use (blue liquids)
Q213Z	CONTROL	**Control Serum**	0.5 mL	1	Solution based on human plasma, containing of known fT3 content, with preservative, ready to use (colourless liquid)
T213Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of fT3 conjugated to the horseradish peroxidase; ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength;
– dry thermostat for +37°C±2°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The fT3 EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The fT3 EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.
- diluted washing solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 14 wells for Calibrators and Control Serum (2 wells for each calibrator (CAL 1-6) and 2 wells for control serum (Q)).

10.2 Dispense **25 µL of Calibrators and Control Serum as well as 25 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

Note: during performing several independent series of tests, Calibrators, and Control Sample should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5								
E	CAL5	CAL5	SAMP6	SAMP6								
F	CAL6	CAL6	SAMP7	SAMP7								
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.3 Add **100 µL of the Conjugate Solution** to all wells.

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6 Add **100 µL of Substrate Solution** to all wells. The introduction of the substrate solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.7 Add **100 µL of Stop Solution** to all wells in the same order as the substrate solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.8 Read the optical density (OD) of the wells at 450nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.9 Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the fT3 concentration in the calibrators pmol/L, (y) – OD versus fT3 concentration (OD 450 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve. Adjust the concentration of CAL1 to an infinitesimally small value, for example, 0.001 pmol/L.

10.10 Determine the corresponding concentration of fT3 in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 1.2, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for fT3. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

NOTE: values of fT3 concentrations in the tested samples that are below the LoD (0.5 pmol/L) and also exceed the value of the upper calibrator (40 pmol/L) should be provided in the following form: «the fT3 concentration of tested sample X is «lower than 0.5 pmol/L» or «higher than 40pmol/L».

Sex, age	Units, pmol/L	
	Lower limit	Upper limit
Healthy donors	2.5	5.8

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

3.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, pmol/L	CV, %
1	4.32	7.44
2	6.87	5.14

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations..

Sample	Concentration, pmol/L	CV, %
1	2,34	7,12
2	3,83	6,41

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, pmol/L	Concentration2, pmol/L	Concentration3, pmol/L	CV, %
1	5.17	5.42	4.78	6.54
2	3.61	3.78	3.45	9.6

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known fT3 concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 2.5–40 pmol/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest fT3 concentration in the serum or plasma sample that is detected by the fT3 EIA kit is no lower than 2.0 pmol/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for fT3 EIA kit is 2.25 pmol/L.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of fT3 with other analytes is shown in the table:

Analyte	Cross-reactivity, %
L-Thyroxin	0,01
D-Thyroxin	0,04

14. REFERENCES

1. Physiology of thyroid hormones. IN: Division of Drugs and Toxicology, American Medical Association: Drug Evaluations Annual 1995. Amer Med Assn, Chicago, 1995, ch 47, pp 1039-1040.
2. Robins J & Rall JE. The Iodine -Containing Hormones. IN Hormones in Blood (2nd ed) 1: 383-490, Gray CH & Bacharach AL (eds) London Academic Press, 1987.
3. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
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LOT _____ DATE _____

	Manufacturer
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REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
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Instruction for use
A solid-phase enzyme immunoassay kit
for the quantitative determination of
free thyroxin in human serum or plasma

fT4 EIA

Catalogue number REF **K214**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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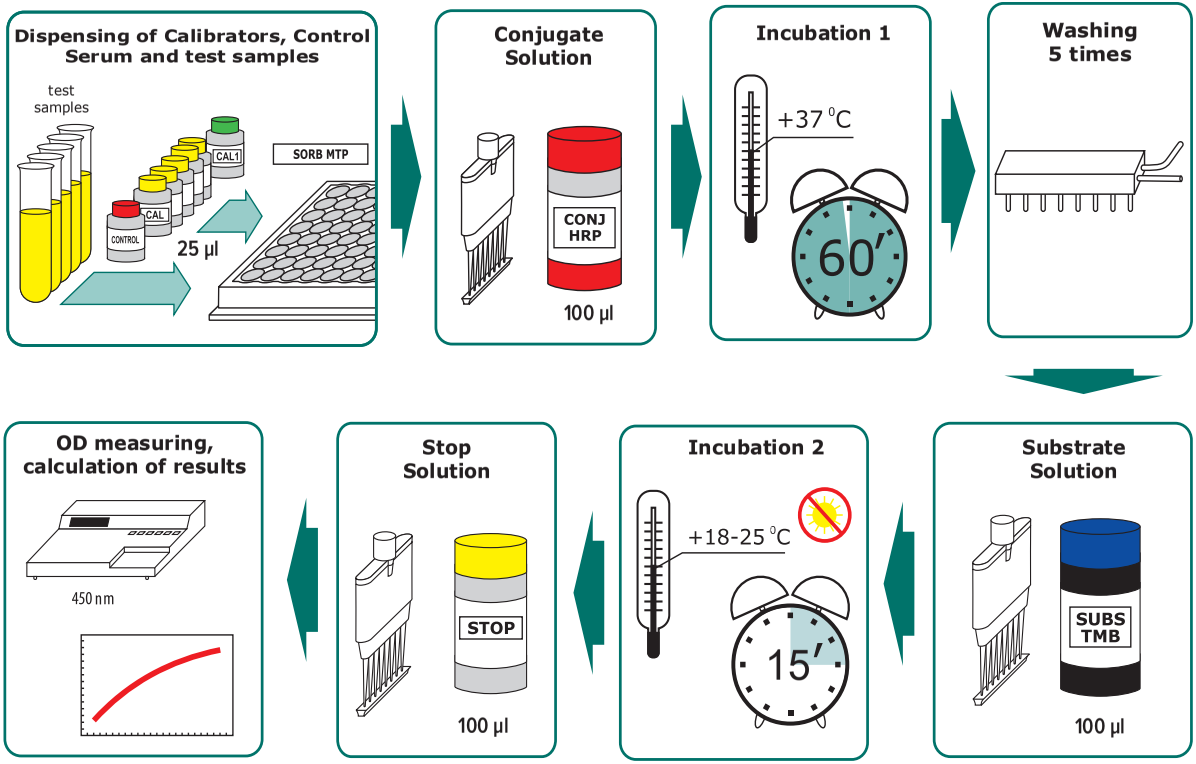
www.polmed.de

ASSAY PROCEDURE



K214

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of free thyroxin in human serum or plasma
fT4 EIA

1. INTENDED USE

The fT4 EIA kit is an enzyme immunoassay, intended for the quantitative determination of free thyroxin in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Thyroid hormones thyroxin (T4) and 3,5,3'-triiodothyronine (T3) exert regulatory influences on growth, differentiation, cellular metabolism and development of skeletal and organ systems. T4 and T3 in blood are found both in free and bound form – mostly, they are bound to thyroxin binding globulin (TBG). Only free forms of T3 and T4 exert hormonal activity also their percentage is very low – 0.3% for T3 and 0.03% for T4.

The concentration of T4 is generally accepted as an index of thyroid function which provide enough information to differentiate between hyper-, hypo- and euthyroidism.

Elevation of total T4 is found in hyperthyroidism, in patients with tumours of pituitary gland, in subjects with elevated TBG level (pregnancy, acute or chronic active hepatitis, estrogen-secreting tumours or estrogen intake, hereditary elevation of TBG), in patients taking oral contraceptives, heroin, methadone, thyroid preparations, TSH, thyroliberin.

Low total T4 is found in hypothyroidism, in patients with panhypopituitarism, in subjects with low TBG level (acromegaly, nephritic syndrome, hypoproteinemia, chronic liver diseases, androgen-secreting tumours, hereditary reduction), in patients taking aminosalicylic and acetylsalicylic acids, cholestyramine, reserpine, potassium iodide, triiodothyronine.

3. TEST PRINCIPLE

Determination of free thyroxin is based on competition principle of the enzyme immunoassay. Microwells plate is coated with specific murine monoclonal antibodies to T4. fT4 conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage fT4 from the specimen competes with the conjugated fT4 for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed.
- during the second stage, the complexes formed due the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. Optical density in the microwell is inversely related to the quantity of the measured fT4 in the specimen of the serum (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of fT4 in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P214Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to T4; ready to use
C214Z	CAL 1	**Calibrator C1**	0.5 mL	1	Solution based on human plasma, free of fT4, with preservative, ready to use (yellow liquid)
C214Z	CAL 2-6	**Calibrators**	0.5 ml	5	Solutions based on human plasma, containing 5; 10; 25, 50 and 100 pmol/L of fT4, with preservative, ready to use (red liquids)
Q214Z	CONTROL	**Control Serum**	0.5 ml	1	Solution based on human plasma, containing of known fT4 content, with preservative, ready to use (colourless liquid)
T214Z	CONJ HRP	**Conjugate Solution**	12 ml	1	Solution of fT4 conjugated to the horseradish peroxidase; ready to use (red liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 ml	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 ml	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 ml	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (1 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm wavelength;
- dry thermostat for +37°C±1°C;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- vortex mixer;
- disposable gloves;
- absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The fT4 EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The fT4 EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.
- diluted washing solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 14 wells for Calibrators and Control Serum (2 wells for each calibrator (CAL 1-6) and 2 wells for control serum (Q)).

10.2 Dispense **25 µL of Calibrators and Control Serum as well as 25 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

Note: during performing several independent series of tests, Calibrators, and Control Sample should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5								
E	CAL5	CAL5	SAMP6	SAMP6								
F	CAL6	CAL6	SAMP7	SAMP7								
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.3 Add **100 µL of the Conjugate Solution** to all wells.

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL

10.6 Add **100 µL of Substrate Solution** to all wells. The introduction of the substrate solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.7 Add **100 µL of Stop Solution** to all wells in the same order as the substrate solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.8 Read the optical density (OD) of the wells at 450nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.9 Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the fT4 concentration in the calibrators pmol/L, (y) – OD versus fT4 concentration (OD 450 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve. Adjust the concentration of CAL1 to an infinitesimally small value, for example, 0.001 pmol/L.

10.10 Determine the corresponding concentration of fT4 in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 1.2, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for fT4. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

NOTE: values of fT4 concentrations in the tested samples that are below the LoD (0.75 pmol/L) and also exceed the value of the upper calibrator (100 pmol/L) should be provided in the following form : «the fT4 concentration of tested sample X is «lower than 0.75 pmol/L» or «higher than 100 pmol/L».

Sex, age	Units, pmol/L	
	Lower limit	Upper limit
Healthy donors		
< 60 yrs	10	25
> 60 yrs	10	21
Pregnancy week		
1st trimester	9	26
2nd trimester	6	21
3rd trimester	6	21

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, pmol/L	CV, %
1	54.4	5.83
2	85.23	3.67

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, pmol/L	CV, %
1	54.36	1.15
2	85.73	3.23

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, pmol/L	Concentration2, pmol/L	Concentration3, pmol/L	CV, %
1	54.59	52.67	60.39	7.19
2	85.23	87.53	85.13	1.58

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known fT4 concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 5-100 pmol/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest fT4 concentration in the serum or plasma sample that is detected by the fT4 EIA kit is no lower than 0.75 pmol/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for fT4 EIA kit is 5 pmol/L.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of fT4 with other analytes is shown in the table:

Analyte	Cross-reactivity, %
L-Thyroxin	100
D-Thyroxin	94
3,3′,5′-Triiodo-L-Thyronine (Reverse T3)	86
3,3′,5-Triiodo-L-Thyronine (T3)	3.3
3,3′,5′-Triiodo-D-Thyronine	1.8
3,3′,5′-Triiodothyropropionic acid	0.6

14. REFERENCES

1. Tietz, N. W., Fundamentals of Clinical Chemistry, 2nd Ed., pg. 602, Sauders Press, Phila., 1976.
2. Horworth, P. J. N., Ward, RL., J. Clin Pathol. 1972; 25:259-62.
3. Sati, C., Chattor, A. J., Watts, N. Fundamentals of Clinical Chemistry. Ed. Tietz, N. W. 3rd Ed., pg. 586. Saunders press Phila. 1987.
4. Lundberg, P. A., Jagenburg, R., Lindstedt, G., Nystrom, E., Clin. Chem. 1982, 28:1241.
5. Melmed, S., Geola, F. L., Reed, A. W., Pekary, A. E., Park, J., Hershmen, J. M., Clin Endocrin. Metabol. 1982, 54; 300.
6. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
7. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
8. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

qa@xema.com.ua

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of dehydroepiandrosterone sulfate in human serum or plasma

DHEAS EIA

Catalogue number REF **K215**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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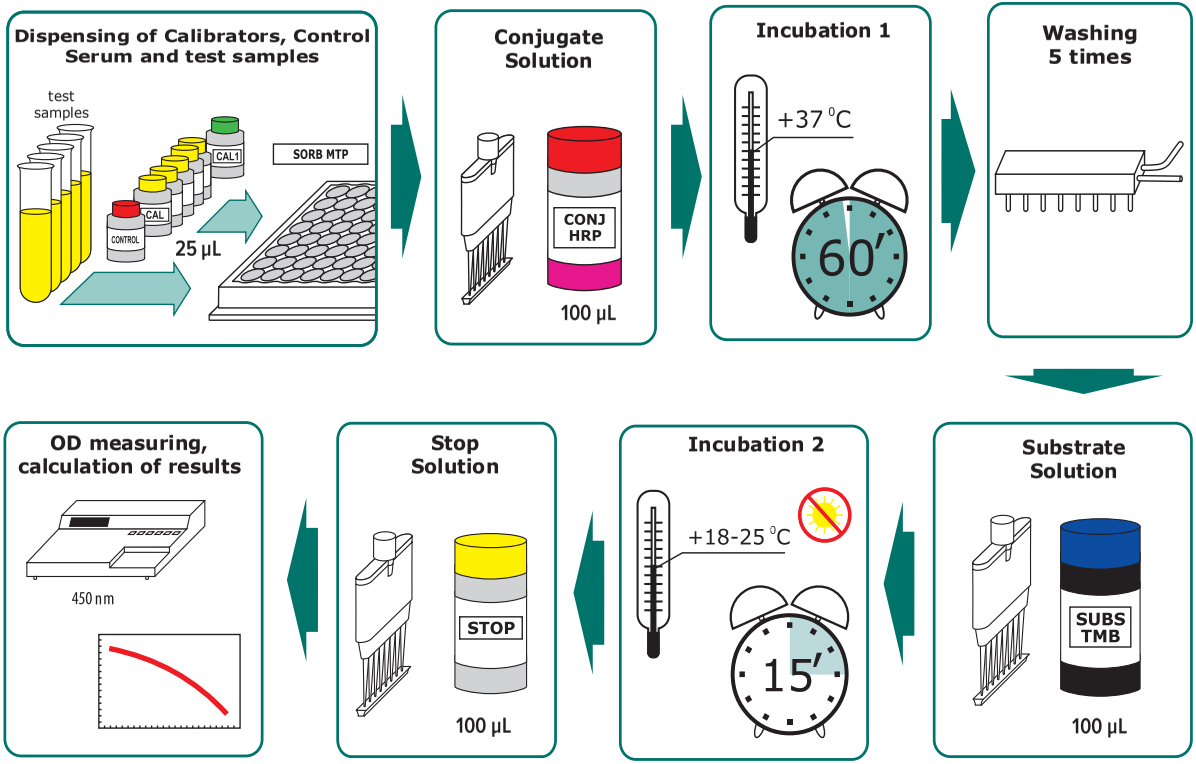
E-mail: info@polmed.de
www.polmed.de

ASSAY PROCEDURE



K215

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of dehydroepiandrosterone sulfate in human serum or plasma
DHEAS EIA

1. INTENDED USE

The DHEAS EIA kit is an enzyme immunoassay, intended for the quantitative determination of dehydroepiandrosterone sulfate in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Dehydroepiandrosterone (DHEA) is an androgen with a MW of 288.4 Dalton secreted in adrenals. The main derivate of DHEA present in human tissue is DHEA sulfate (DHEAS). Since birth, DHEAS serum concentration is increasing continuously showing a pronounced peak after puberty and maximal levels at the age of 20. After that, serum DHEAS level continuously decreases. As DHEAS is the main component of 17-ketosteroids in serum, this test may substitute for column tests for determination of 17-ketosteroids in urine.

Elevated DHEAS concentrations are found in adrenogenital syndrome, hirsutism, acne, benign hyperplasia of adrenals and adrenal tumors, Stein-Leventhal syndrome, polycystic ovary syndrome.

Decreased levels of DHEAS are found in hyperlipidemia, psychotic states, psoriasis, adrenal insufficiency.

3. TEST PRINCIPLE

Determination of the DHEAS is based on competition principle of the enzyme immunoassay. Microwells plate is coated with specific rabbit polyclonal to DHEAS-antibodies. DHEAS conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage DHEAS from the specimen competes with the conjugated DHEAS for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed.
- during the second stage, the complexes formed due the reaction with the chromogen 3,3’,5,5’-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. Optical density in the microwell is inversely related to the quantity of the measured DHEAS in the specimen of the serum (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of DHEAS in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P215Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with rabbit polyclonal antibodies to DHEAS; ready to use
C215Z	CAL 1	**Calibrator C1**	0.5 mL	1	Solution based on human plasma, free of DHEAS, with preservative, ready to use (yellow liquid)
C215Z	CAL 2-6	**Calibrators**	0.5 mL	5	Solutions based on human plasma, containing 0,1; 0.3; 1; 3 and 10 µg/mL of DHEAS, with preservative, ready to use (blue liquids)
Q215Z	CONTROL	**Control Serum**	0.5 mL	1	Solution based on human plasma, containing of known DHEAS content, with preservative, ready to use (colourless liquid)
T215Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of DHEAS conjugated to the horseradish peroxidase; ready to use (magenta liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm wavelength;
- dry thermostat for +37°C±2°C;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- vortex mixer;
- disposable gloves;
- absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The DHEAS EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The DHEAS EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;

  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed
- diluted washing solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 14 wells for Calibrators and Control Serum (2 wells for each calibrator (CAL 1-6) and 2 wells for control serum (Q)).

10.2 Dispense **25 µL of Calibrators and Control Serum as well as 25 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

Note: during performing several independent series of tests, Calibrators, and Control Sample should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5								
E	CAL5	CAL5	SAMP6	SAMP6								
F	CAL6	CAL6	SAMP7	SAMP7								
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.3 Add **100 µL of the Conjugate Solution** to all wells.

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL

10.6 Add **100 µL of Substrate Solution** to all wells. The introduction of the substrate solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.7 Add **100 µL of Stop Solution** to all wells in the same order as the substrate solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.8 Read the optical density (OD) of the wells at 450nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.9 Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the DHEAS concentration in the calibrators µg/mL, (y) – OD versus DHEAS concentration (OD 450 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve. Adjust the concentration of CAL1 to an infinitesimally small value, for example, 0.001 µg/mL.

10.10 Determine the corresponding concentration of DHEAS in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 1.2, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

12.1 Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for DHEAS. Based on data obtained by XEMA, the following normal range is recommended (see below).

NOTE: values of DHEAS concentrations in the tested samples that are below the LoD (0.025 µg/mL) and also exceed the value of the upper calibrator (10 µg/mL) should be provided in the following form : «the DHEAS concentration of tested sample X is «lower than 0.025 µg/mL» or «higher than 10 µg/mL».

12.2 The calibrators concentration values of the DHEAS EIA kit are expressed in µg/mL. To calculate concentrations in µmol/L, the received concentration value in µg/mL shall be multiplied by 2.6.

1 µg/mL = 2.6 µmol/L

Sex, age	Units, µg/mL		Units alternative, µmol/L	
	Lower limit	Upper limit	Lower limit	Upper limit
Males				
newborn	1.08	4.06	2.81	10.56
1 month-5 yrs	0.01	0.41	0.03	1.07
6-9 yrs	0.03	1.45	0.07	3.77
10-11 yrs	0.12	1.15	0.31	2.99
12-17 yrs	0.2	5.55	0.52	14.43
18-30 yrs	1.25	6.19	3.25	16.09
31-50 yrs	0.59	4.52	1.53	11.75
51-60 yrs	0.2	4.13	0.52	10.74
>61 yrs	0.1	2.35	0.26	6.11
Females				
newborn	0.1	2.48	0.26	6.45
1 month-5 yrs	0.05	0.55	0.13	1.43
6-9 yrs	0.03	1.4	0.07	3.64
10-11 yrs	0.15	2.6	0.39	6.76
12-17 yrs	0.2	5.35	0.52	13.91
18-30 yrs	0.29	7.81	0.75	20.31
31-60 yrs	0.12	3.79	0.31	9.85
post menopausal	0.3	2.6	0.78	6.76
Pregnancy week				
1st trimester	0.38	3.6	0.99	9.36
2nd trimester	0.42	3.0	1.09	7.8
3rd trimester	0.32	2.5	0.83	6.5

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, µg/mL	CV, %
1	4.02	5.9
2	3.38	7.34

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, µg/mL	CV, %
1	2.49	6.12
2	4.23	7.41

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration, µg/mL	Concentration, µg/mL	Concentration, µg/mL	CV, %
1	1.98	1.89	2.03	11.45
2	1.69	1.78	1.64	13.6

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known DHEAS concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 0.1-10 µg/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest DHEAS concentration in the serum or plasma sample that is detected by the DHEAS EIA kit is no lower than 0.05 µg/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for DHEAS EIA kit is 0.08 µg/mL.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of DHEAS with other analytes is shown in the table:

Analyte	Cross-reactivity, %
DHEA	50
other steroids	<0,01

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10. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
11. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

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LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

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LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

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Instruction for use
A solid-phase enzyme immunoassay kit
for the quantitative determination of
free testosterone in human serum or plasma

free Testosterone EIA

Catalogue number REF **K219**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

CE

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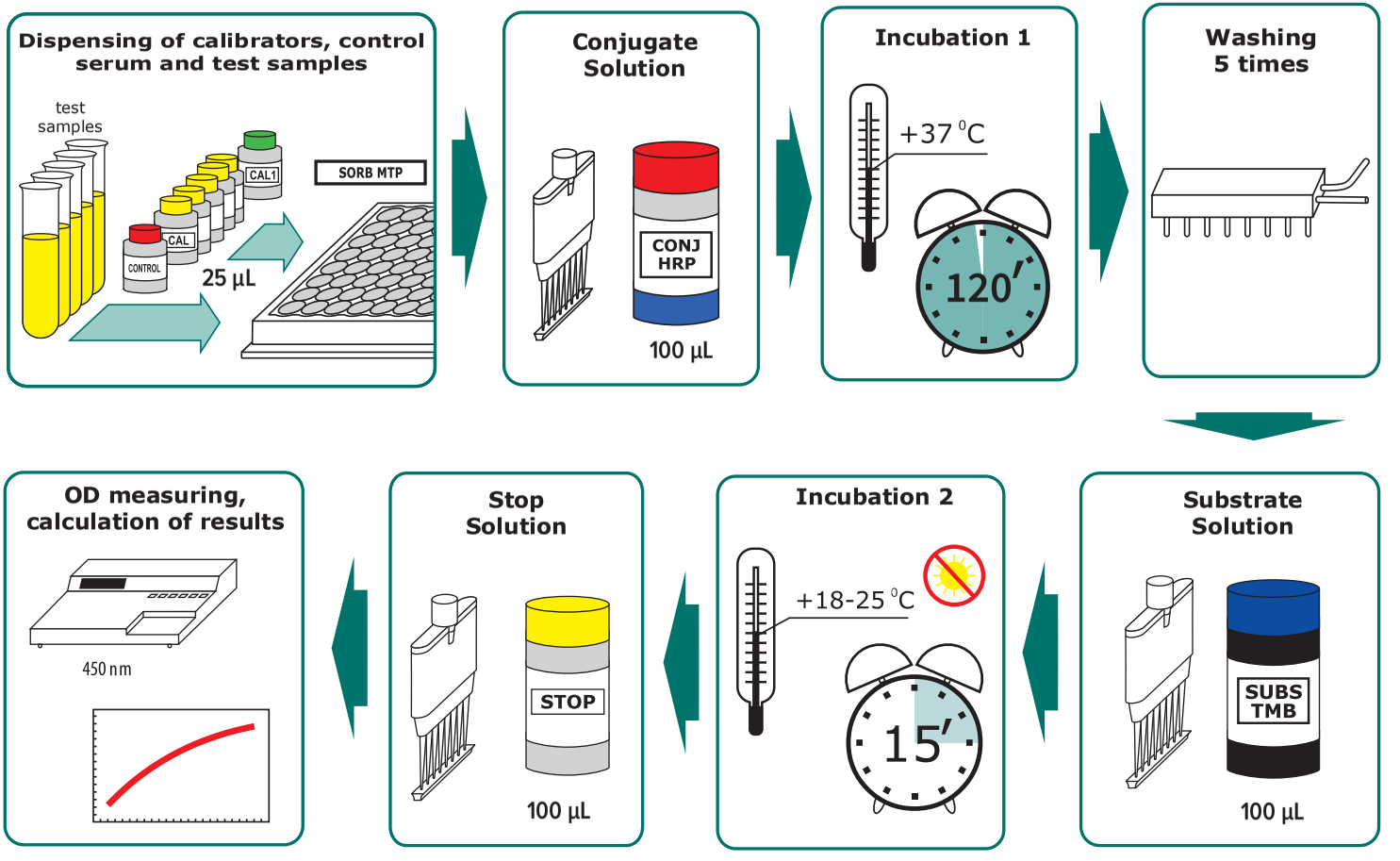
ASSAY PROCEDURE



K219

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of free testosterone in human serum or plasma
free Testosterone EIA

1. INTENDED USE

The free Testosterone EIA kit is an enzyme immunoassay, intended for the quantitative determination of free testosterone in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Testosterone is a steroid with a MW of 288.4 Dalton. The main sites of testosterone secretion are Leidig cells in interstitial tissue of testicles in men. In women testosterone is secreted in the adrenals and is controlled by luteinizing hormone. Testosterone stimulates development of male genital organs and formation of secondary sexual features.

In males, testosterone secretion undergoes circadian rhythms with maximal concentrations seen in the morning (6 am) and minimal – in the evening (8 pm). In females, testosterone secretion is regulated by menstrual cycle with maximal levels found in luteinic phase and during ovulation.

Free testosterone is a fraction of serum testosterone not bound to sex-binding globulin hormones (SHBG) and with albumin. Free testosterone makes up 2 - 3% of total testosterone.

Biologically active is only testosterone is free and bound to albumin («bioavailable testosterone»). The level of «bioavailable testosterone» reflects the amount functionally active testosterone in the body.

3. TEST PRINCIPLE

Determination of the free testosterone is based on competition principle of the enzyme immunoassay. Microwells plate is coated with specific to free testosterone murine monoclonal antibodies. Testosterone conjugated to the horseradish peroxidase is used as enzyme conjugate.

The analysis procedure includes two stages of incubation:

- during the first stage free testosterone from the specimen competes with the conjugated testosterone for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed.
- during the second stage, the complexes formed due the reaction with the chromogen 3,3’,5,5’-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. Optical density in the microwell is inversely related to the quantity of the measured free testosterone in the specimen of the serum (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of free testosterone in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P219Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to free testosterone, ready to use
C219Z	CAL 1	**Calibrator C1**	0,5 mL	1	Solution based on human plasma, free of testosterone, with preservative, ready to use (yellow liquid)
C219Z	CAL 2-6	**Calibrators**	0,5 mL	5	Solutions based on human plasma, containing 0.2; 1; 4; 20 and 100 pg/mL of free testosterone, with preservative, ready to use (red liquids)
Q219Z	CONTROL	**Control serum**	0,5 mL	1	Solution based on human plasma, containing of known free testosterone content, with preservative, ready to use (colourless liquid)
T219Z	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of testosterone conjugated to the horseradish peroxidase, ready to use (blue liquid
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution, ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid, ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (1 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm wavelength;
- dry thermostat for 37°C±1°C;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- vortex mixer;
- disposable gloves;
- absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The free Testosterone EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The free Testosterone EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months.

NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.

- diluted washing solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

10. ASSAY PROCEDURE

10.1. Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 14 wells for Calibrators and Control serum (2 wells for each Calibrators (CAL 1-6) and 2 wells for Control Serum (Q)).

10.2. Dispense **25 µL of Calibrators and Control Serum as well as 25 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

Note: during performing several independent series of tests, Calibrators, and Control Sample should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5	SAMP13	SAMP13						
E	CAL5	CAL5	SAMP6	SAMP6	SAMP14	SAMP14						
F	CAL6	CAL6	SAMP7	SAMP7	SAMP15	SAMP15						
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.3. Add **100 µL of the Conjugate Solution** to all wells.

10.4. Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **120 minutes at +37°C**.

10.5. At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6. Add **100 µL of Substrate Solution** to all wells. The introduction of the substrate solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.7. Add **100 µL of Stop Solution** to all wells in the same order as the substrate solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.8. Read the optical density (OD) of the wells at 450nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.9. Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the free testosterone concentration in the Calibrators pg/mL, (y) – OD versus free testosterone concentration (OD 450 nm). Manual or computerized data reduction is applicable at this stage. For the algorithm calculation (approximation) of the calibration curve, using the interval (segment-linear, point-to-point) method is recommended. Adjust the concentration of CAL1 to an infinitesimally small value, for example, 0.001 pg/mL.

10.10. Determine the corresponding concentration of free testosterone in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 1.2, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on the results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for free testosterone. Based on data obtained by XEMA, the following normal range is recommended (see below).

NOTE: values of free testosterone concentrations in the tested samples that are below the LoD (0.06 pg/mL) and also exceed the value of the upper calibrator (100 pg/mL) should be provided in the following form: «the free testosterone concentration of tested sample X is «lower than 0.06 pg/mL» or «higher than 100 pg/mL» .

Sex, age	Units, pg/mL	
	Lower limit	Upper limit
Males	4.5	42
Females	-	4.1
Females post menopausal	0.1	4.7

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, pg/mL	CV, %
1	10.4	3.46
2	5.6	4.39

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, pg/mL	CV, %
1	10.2	2.33
2	5.1	7.43

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, pg/mL	Concentration2, pg/mL	Concentration3, pg/mL	CV, %
1	10.5	10.8	10.6	1.44
2	5.4	5.5	5.7	2.76

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known free testosterone concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 0.2-100 pg/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest free testosterone concentration in the serum or plasma sample that is detected by the free Testosterone EIA kit is no lower than 0.06 pg/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for free Testosterone EIA kit is 0.2 pg/mL.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of free testosterone with other analytes is shown in the table:

Analyte	Cross-reactivity, %
5-alpha-dehydrotestosterone	16
Androstendiol	1.0
Androstendione	0.4
Androstendione	< 0.1
Dehydroepiandrosterone	< 0.1
Progesterone	< 0.1
Estradiol, Estriol	< 0.01
Cortisol,Pregnenolone	< 0.01

14. REFERENCES

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SAMPLES IDENTIFICATION PLAN

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F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

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Instruction for use
A solid-phase enzyme immunoassay kit
for the quantitative determination of
CA 125 in human serum or plasma

CA 125 EIA

On the website www.xema.com.ua is available a calculator for calculating the risk of ovarian cancer based on the results of the testing CA 125 and HE4 antigens using EIA kits manufactured by our company.

Catalogue number REF **K222**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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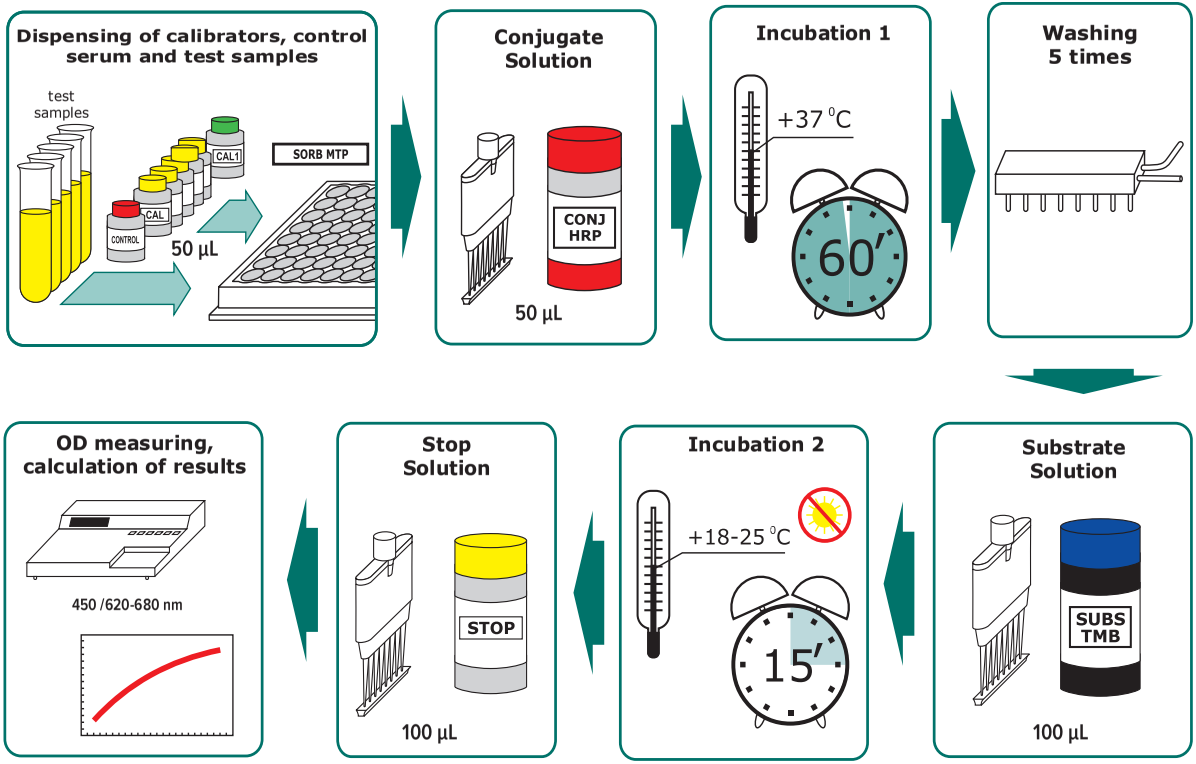
ASSAY PROCEDURE



K222

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of CA 125 in human serum or plasma
CA 125 EIA

1. INTENDED USE

The CA 125 EIA kit is an enzyme immunoassay, intended for the quantitative determination of CA 125 in human serum or plasma.

Quantitative determination of CA 125 in serum (plasma) is used to monitor patients with ovarian adenocarcinomas.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

CA 125 is an antigen (an epitope) associated with ovarian carcinoma and some other tumors. The CA125 epitope is found on a heterogeneous group of glycoproteins with a high molecular weight (MW 200.000 to over 1.000.000). In a high percentage of cases, CA 125 is increased in adenocarcinomas ovaries, with the exception of mucinous and granulosa cell histology forms. In addition, CA125 is detectable in some fetal tissues and in adult tissues in the epithelium of the phallopian tubes, apocrine sweat glands, breast glands, endometrium and endocervix. Elevated serum concentrations of CA125 are found in most patients with epithelial ovarian cancer, including those with stage 1 disease. CA125 determination is useful for therapy control and follow-up of ovarian cancer patients treated by any type of therapy. However, the CA125 values obtained should always be interpreted in the context of the results obtained by other clinical procedures.

Internal data obtained by XEMA suggest that serial determination of CA125 may be helpful for diagnosis of adenocarcinoma development in fibrotic lung tissue in patients with interstitial lung diseases. In a present test system, monoclonal antibodies X306 (epitope group A) is used to capture the antigen, and monoclonal antibodies X52 (epitope group B) are used as a tracer. The epitope specificity of both antibodies were confirmed by an independent expert group (TD1 workshop 2000, International Society of Oncodevelopmental Biology and Medicine).

Determination of CA125 is not suitable for early diagnosis of malignancies because elevated CA125 values may also be found in patients with uterine carcinoma, hepatoma and pancreatic adenocarcinoma as well as in non-malignant conditions such as liver cirrhosis, interstitial lung diseases.

WARNING! This kit is intended for use only with serum or plasma human blood. When analyzing other types of samples – for example, ascitic fluid, pleural effusions or culture supernatants may be obtained false results.

3. PRINCIPLE OF THE TEST

The determination of the CA 125 is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to human CA 125. Second antibodies – murine monoclonal antibodies to human CA125 conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage CA 125 from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized CA 125;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured CA 125 in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density on the content of CA 125 in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P222Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to human CA125; ready to use
C222Z	CAL 1	**Calibrator C1**	6 mL	1	Solution based on tris buffer (pH 7.2-7.4), free of human CA125, with preservative, ready to use (yellow liquid)
C222Z	CAL 2-6	**Calibrators**	0.6 mL	5	Solutions based on tris buffer (pH 7.2-7.4), containing 25; 50; 100, 200 and 400 U/mL of human CA 125, ready to use (red liquids)
Q222Z	CONTROL	**Control Serum**	0.6 mL	1	Solution based on human serum, containing of known human CA 125 content, with preservative, ready to use (colourless liquid)
T222Z	CONJ HRP	**Conjugate Solution**	7 mL	1	Solution of murine monocnoclonal antibodies to CA 125 conjugated to the horseradish peroxidase; ready to use (red liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (1 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength or 450\620-680 nm;
– dry thermostat for 37°C±1°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The CA 125 EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The CA 125 EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;

  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 400 U/mL, additionally dilute this sample accordingly, using (Calibrator C1). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of the blood serum (plasma) sample.

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 14 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-6) and 2 wells for Control Serum (Q)).

10.2 If necessary, dilute the test samples as described in 9.4.

10.3 Dispense **50 µL of Calibrators and Control Serum as well as 50 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5								
E	CAL5	CAL5	SAMP6	SAMP6								
F	CAL6	CAL6	SAMP7	SAMP7								
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.4 Add **50 µL of Conjugate Solution** to all wells.

10.5 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.6 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the wash solution volume can be increased to 350 µL.

10.7 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.8 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.9 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the Stop Solution. Set photometer blank on CAL1.

10.10 Plot a calibration curve in linear coordinates: (x) is the CA 125 concentration in the calibrators U/mL, (y) – OD versus CA 125 concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.11 Determine the corresponding concentration of CA 125 in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on the results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for CA 125. Based on data obtained by XEMA, the following normal range is recommended (see below).

NOTE: values of CA 125 concentrations in the tested samples that are below the LoD (0.25 U/mL) and also exceed the value of the upper Calibrator (400 U/mL) should be provided in the following form : «the CA 125 concentration of tested sample X is «lower than 0.25 U/mL» or «higher than 400 U/mL».

Sex, age	Одиниці, U/mL	
	Lower limit	Upper limit
Males	-	35
Females	-	35
Pregnancy week		
1st trimester	-	60
2nd trimester	-	150
3rd trimester	-	200
Lactation	-	80

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, U/mL	CV, %
1	57.4	7.83
2	259	1.67

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, U/mL	CV, %
1	56.34	1.75
2	258.47	5.63

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, U/mL	Concentration2, U/mL	CV, %
1	56.6	57.89	1.59
2	259.4	261.75	0.64

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the mezhurand. The bias was calculated for each sample and it was determined whether it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known CA 125 concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 25–400 U/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest CA 125 concentration in the serum or plasma sample that is detected by the CA 125 EIA kit is no lower than 0.25 U/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for CA 125 EIA kit is 25 U/mL.

13.1.5 Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 400 U/mL.

13.1.6 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of CA 125 with other analytes is shown in the table:

Analyte	Cross-reactivity, %
CEA	< 0.1
CA 19-9	< 0.1
CA 15-3	< 0.1

14. REFERENCES

1. Engall, E., Methods in Enzymology, Volume 70, Van Vunakis, H. and Langone, J. J. (eds.), Academic Press, New York, 419- 492. (1980).
2. Kenemans P, Yedema CA, Bon GG, von Mensdorff-Pouilly S. Ca125 in gynecological pathology a review. Eur J Obstet Gynecol 1993; 49:115-124
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5. Welander CE. What do CA 125 and other antigens tell us about ovarian cancer biology. Acta Obstet Gynecol Scand Sup 1992; 155:85-93
6. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
7. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
8. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

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Instruction for use
A solid-phase enzyme immunoassay kit
for the quantitative determination of
CA 19-9 in human serum or plasma

CA 19-9 EIA

Catalogue number REF **K223**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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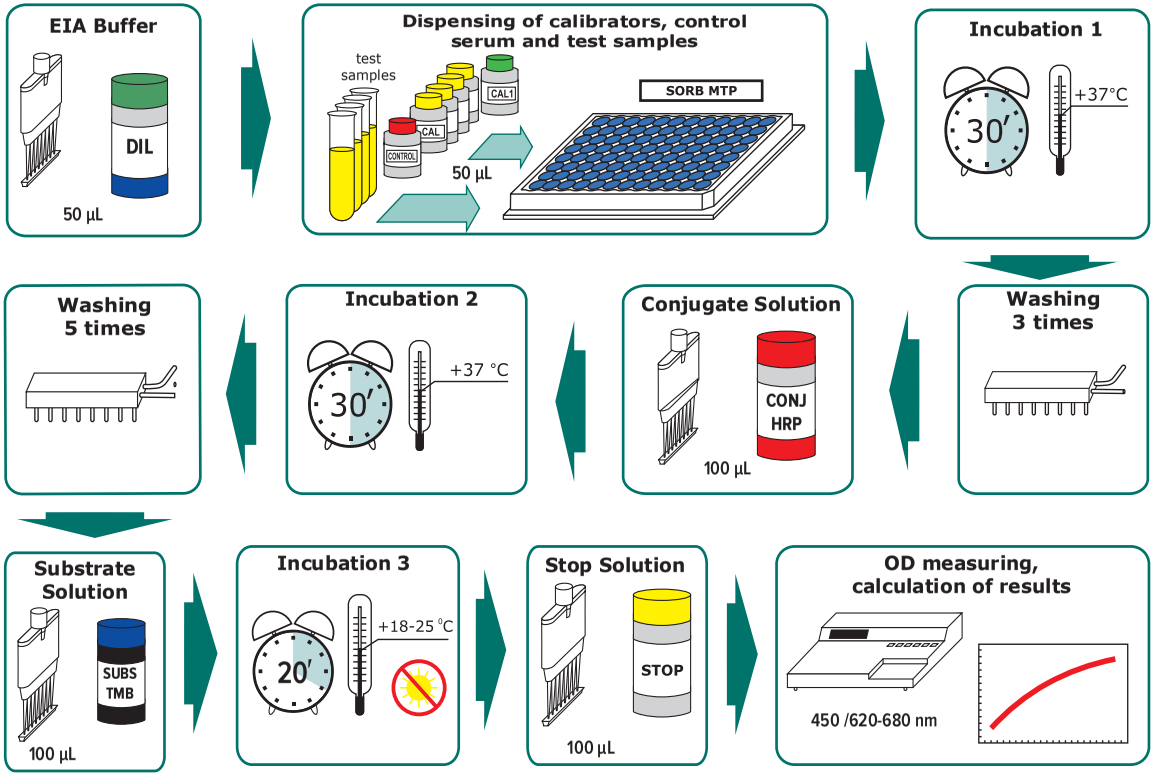
ASSAY PROCEDURE



K223

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of CA 19-9 in human serum or plasma
CA 19-9 EIA

1. INTENDED USE

The CA 19-9 EIA kit is an enzyme immunoassay, intended for the quantitative determination of CA 19-9 concentration in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

CA 19-9 or Sialyl Lewis antigen is an antigen (an epitope) associated with tumours of the gastrointestinal tract, such as pancreatic, liver, stomach and colorectal carcinoma. Quantitative determination of CA19-9 in serum and plasma is helpful in monitoring of patients where such tumours have been diagnosed, especially together with determination of carcinoembryonic antigen. However, the output antigen from the circulation depends on the patency of the bile ducts, therefore cholestasis may cause an inadequate increase in the level of antigen in the serum. Some individuals have lack of the enzyme responsible for synthesis of sialyl-Lewis antigen and therefore cannot respond by antigen elevation even to progressive tumour growth. However, the CA 19-9 values obtained should always be interpreted in the context of the results obtained by other clinical procedures.

3. TEST PRINCIPLE

The determination of the CA 19-9 is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to human CA 19-9. Second antibodies – murine monoclonal antibodies to human CA 19-9 conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes tree stages of incubation:

- during the first stage CA 19-9 from the specimen is captured by the antibodies coated onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized CA 19-9, fixed in the formed at the previous stage complexes;
- during the third stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured CA 19-9 in the serum specimen (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of CA 19-9 in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P223Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to human CA 19-9; ready to use
C223Z	CAL 1	**Calibrator C1**	6 mL	1	Solution based on phosphate buffer (pH 7.2-7.4), free of CA 19-9, with preservative, ready to use (colourless liquid)
C223Z	CAL 2-5	**Calibrators**	0,8 mL	4	PSolution based on phosphate buffer (pH 7.2-7.4), containing 12; 60; 120 and 240 U/mL CA 19-9, ready to use (red liquids)
Q223Z	CONTROL	**Control Serum**	0,8 mL	1	Solution based on human serum, containing of known CA 19-9 content, with preservative, ready to use (colourless liquid)
T223Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of murine monoclonal antibodies to human CA 19-9 conjugated to the horseradish peroxidase; ready to use (red liquid)
S011Z	DIL	**EIA Buffer**	14 mL	1	Buffer solution with detergent and preservative, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	2	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (3 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength or 450\620-680 nm;
– dry thermostat for +37°C±2 °C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

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- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The CA 19-9 EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The CA 19-9 EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- EIA Buffer, Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;

  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

  Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing solution preparation

Add the contents of the 22 mL washing solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the washing solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 240 U/mL, additionally dilute this sample accordingly, using (Calibrator C1). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of the blood serum (plasma) sample

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2 If necessary, dilute the test samples as described in 9.4

10.3 Dispense **50 µL of EIA Buffer** to all wells.

10.4 Dispense **50 µL of Calibrators and Control Serum as well as 50 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5								
D	CAL4	CAL4	SAMP6	SAMP6								
E	CAL5	CAL5	SAMP7	SAMP7								
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.5 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **30 minutes at +37 °C**.

10.6 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 3 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.7 Add **100 µL of Conjugate Solution** to all wells.

10.8 Cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.9 At the end of the incubation period, aspirate and wash each well 5 times as described in 10.6.

10.10 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18…+25°C) for 20 minutes**.

10.11 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.12 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the stop solution. Set photometer blank on CAL1.

10.13 Plot a calibration curve in linear coordinates: (x) is the concentration of CA 19-9 in the Calibrators U/mL, (y) – OD versus concentration of CA 19-9 (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.14 Determine the corresponding concentration of CA 19-9 in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on the results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for CA 19-9. Based on data obtained by XEMA, the following normal range is recommended (see below).

NOTE: values of CA 19-9 concentrations in the tested samples that are below the LoD (1.0 U/mL) and also exceed the value of the upper calibrator (240 U/mL) should be provided in the following form : «the CA 19-9 concentration of tested sample X is «lower than 1.0 U/mL» or «higher than 240 U/mL»

Sex, age	Units, U/mL	
	Lower limit	Upper limit
Healthy donors	-	35

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

3.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, U/mL	CV, %
1	12.3	6.2
2	62.5	3.8

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, U/mL	CV, %
1	12.27	6.3
2	63.87	5.2

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, U/mL	Concentration2, U/mL	Concentration3, U/mL	CV, %
1	12.32	12.02	12.81	3.2
2	63.71	64.56	62	2.1

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the mezhurand. The bias was calculated for each sample and it was determined whether it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known CA 19-9 concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 12–120 U/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest CA 19-9 concentration in the serum or plasma sample that is detected by the CA 19-9 EIA kit is no lower than 1,0 U/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for CA 19-9 EIA kit is 12 U/mL.

13.1.6 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of CA 19-9 with other analytes is shown in the table:

Analyte	Cross-reactivity, %
CEA	< 0.1
CA 125	< 0.1
CA 15-3	< 0.1

14. REFERENCES

1. Glenn, J., Steinberg, W.M., Kurtzman, S.H., et at. Evaluation of the utility of a radioimmunoassay for serum CA 19-9 level in patients before and after treatment of carcinoma of the pancreas. J. Clin. Oncol. 1988; 6:462...+8.
2. Hayakawa, T., Kondo, T., Shibata, T. et al. Sensitive serum markers for detecting pancreatic cancer. Cancer 1988; 61:1827-31.
3. Koprowski, H., Herly, M., Steplewski, Z., et al. Specific antigen in serum of patients with colon carcinoma. Science 1981; 212:53-5.
4. Malesci, A., Tommasini, M.A., Bonato, C. et al. Determination of CA19-9 antigen in serum and pancreatic juice for differential diagnosis of pancreatic adenocarcinoma from chronic pancreatitis. Gastroenteroglogy 1987; 92:60-7
5. Safi, F, Roscher, R., Bittner, R., et al. High sensitivity and specificity of CA 19-9 for pancreatic carcinoma in comparison to chronic pancreatitis. Serological and immunohistochemical findings. Pancreas 1987; 2:398-403
6. Steinberg, W. The clinical utility of CA 19-9 tumor associated antigen. American J. of Gastroenterology 1990; 85:350-355.
7. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
8. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
9. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81).

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

qa@xema.com.ua

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of carcinoembryonic antigen in human serum or plasma

CEA EIA

Catalogue number REF **K224**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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EC REP

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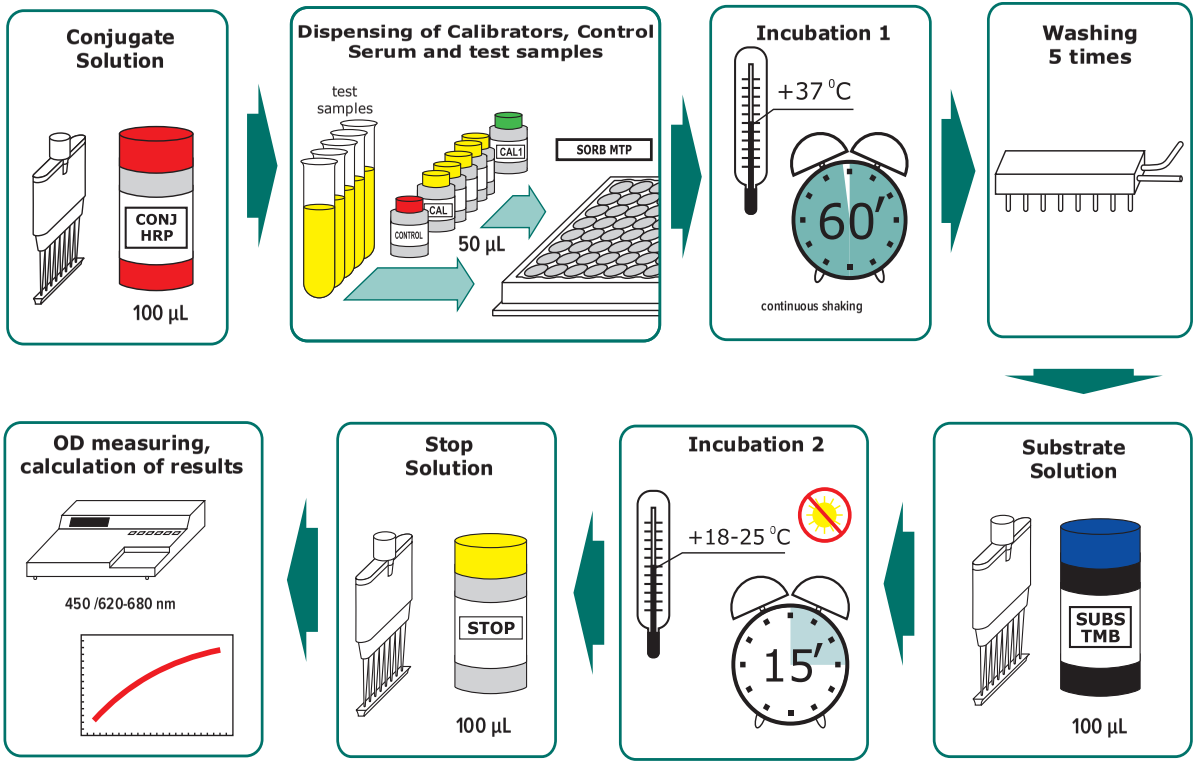
www.polmed.de

ASSAY PROCEDURE



K224

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of carcinoembryonic antigen in human serum or plasma
CEA EIA

1. INTENDED USE

The CEA EIA kit is an enzyme immunoassay, intended for the quantitative determination of carcinoembryonic antigen in human serum or plasma.

Quantitative determination of carcinoembryonic antigen (CEA) in blood serum (plasma) is used as an auxiliary method of early diagnosis, monitoring of tumors of the gastrointestinal tract, breast, lung and other adenocarcinomas, as well as assessment of the effectiveness of the therapy for all population groups.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Carcinoembryonic antigen (CEA) represents a family of heavily glycosylated glycoproteins with MW 180–200 kDa which is expressed and secreted by normal human gastrointectinal mucosa. An increase of the level of CEA in the blood serum may precede recurrence of colon or rectal cancer, which is registered in an average of 4-6 months before the development of clinical manifestations of relapse. Although up to 30% of patients with cancer relapse of this localization do not have elevated levels of CEA in the serum, periodic determining the concentration of CEA is important for monitoring patients after surgery - an increase in the level of CEA indicates a recurrence of cancer. Increase the level of CEA is noted in a number of other epithelial tumors, including carcinoma of the breast, stomach, bronchi, pancreas, esophagus, ovaries and endometrium. Determining the content of the CEA is of the greatest importance in the evaluation effectiveness of anticancer therapy (chemo-, radio- or immunotherapy), as well as during follow-up of patients after surgical removal of tumors for the purpose timely detection of relapse.In blood circulation, there are the substances showing high degree of similarity to CEA (NCA, NCA2); this fact requires the use of highly specific anti-CEA reagents. In a present test-system, we use for capturing CEA the monoclonal antibody 3C6. Due to high prevalence of serum CEA elevation in benign diseases (mucosal inflammations), this test system is not recommended for screening for malignant tumours.

3. PRINCIPLE OF THE TEST

The determination of the CEA is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to human CEA. Second antibodies – murine monoclonal antibodies to human CEA conjugated to the horseradish peroxidase is used as enzyme conjugate.

The analysis procedure includes two stages of incubation:

- during the first stage CEA from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized CEA;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3’,5,5’-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured CEA in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density on the content of CEA in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P224Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to human CEA; ready to use
C224Z	CAL 1	**Calibrator C1**	6 mL	1	Solution based on tris buffer (pH 7.2-7.4), free of human CEA, with preservative, ready to use (yellow liquid)
C224Z	CAL 2-6	**Calibrators**	0.8 mL	5	Solution based on tris buffer (pH 7.2-7.4), containing 2; 4; 8; 32 and 64 ng/mL of CEA, with preservative, ready to use (red liquids)
Q224Z	CONTROL	**Control Serum**	0.8 mL	1	Solution based on human serum, containing of known CEA content, with preservative, ready to use (colourless liquid)
T224Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of murine monocnoclonal antibodies to human CEA conjugated to the horseradish peroxidase; ready to use (red liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength or 450\620-680 nm;
– thermostat shaker maintaining a speed of 300 rpm and temperature of +37°C ±3°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLE

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The CEA EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The CEA EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;

  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 64 ng/mL, additionally dilute this sample accordingly, using (Calibrator C1). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of the blood serum (plasma) sample

Do not dilute Control Serum and Calibrators!

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 14 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-6) and 2 wells for Control Serum (Q)).

10.2 If necessary, dilute the test samples as described in 9.4.

10.3 Dispense **100 µL of Conjugate Solution** to all wells.

10.4 Dispense **50 µL of Calibrators and Control Serum as well as 50 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5								
E	CAL5	CAL5	SAMP6	SAMP6								
F	CAL6	CAL6	SAMP7	SAMP7								
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.5 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C with continuous shaking 300 rpm**.

10.6 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the wash solution volume can be increased to 350 µL.

10.7 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.8 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.9 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.10 Plot a calibration curve in linear coordinates: (x) is the CEA concentration in the calibrators ng/mL, (y) – OD versus CEA concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.11 Determine the corresponding concentration of CEA in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for CEA. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

NOTE: values of CEA concentrations in the tested samples that are below the LoD (0.5 ng/mL) and also exceed the value of the upper Calibrator (64 ng/mL) should be provided in the following form : «the CEA concentration of tested sample X is «lower than 0.5 ng/mL» or «higher than 64ng/mL».

Sex, age	Units, ng/mL	
	Lower limit	Upper limit
Non-smokers	-	5
Smokers	-	10

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, ng/mL	CV, %
1	36.64	4.62
2	12.43	7.29

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, ng/mL	CV, %
1	11.56	8.91
2	5.44	10.28

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, ng/mL	Concentration2, ng/mL	Concentration3, ng/mL	CV, %
1	3.67	3.41	3.59	11.6
2	7.51	7.37	7.69	8.7

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the mezhurand. The bias was calculated for each sample and it was determined whether it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known CEA concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 2-8 ng/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest CEA concentration in the serum or plasma sample that is detected by the CEA EIA kit is no lower than 0.5 ng/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for CEA EIA kit is 1.0 ng/mL.

13.1.5 Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 500 ng/mL.

13.1.6 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of CEA with other analytes is shown in the table:

Analyte	Cross-reactivity, %
CA 125	< 0.1
CA 19-9	< 0.1
CA 15-3	< 0.1

14. REFERENCES

1. Hammarstrom S. The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. Semin Cancer Biol 1999; 9:67-81.
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4. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
5. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
6. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

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LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

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Instruction for use
A solid-phase enzyme immunoassay kit
for the quantitative determination of
alpha-fetoprotein in human serum or plasma

AFP EIA

Catalogue number REF **K225**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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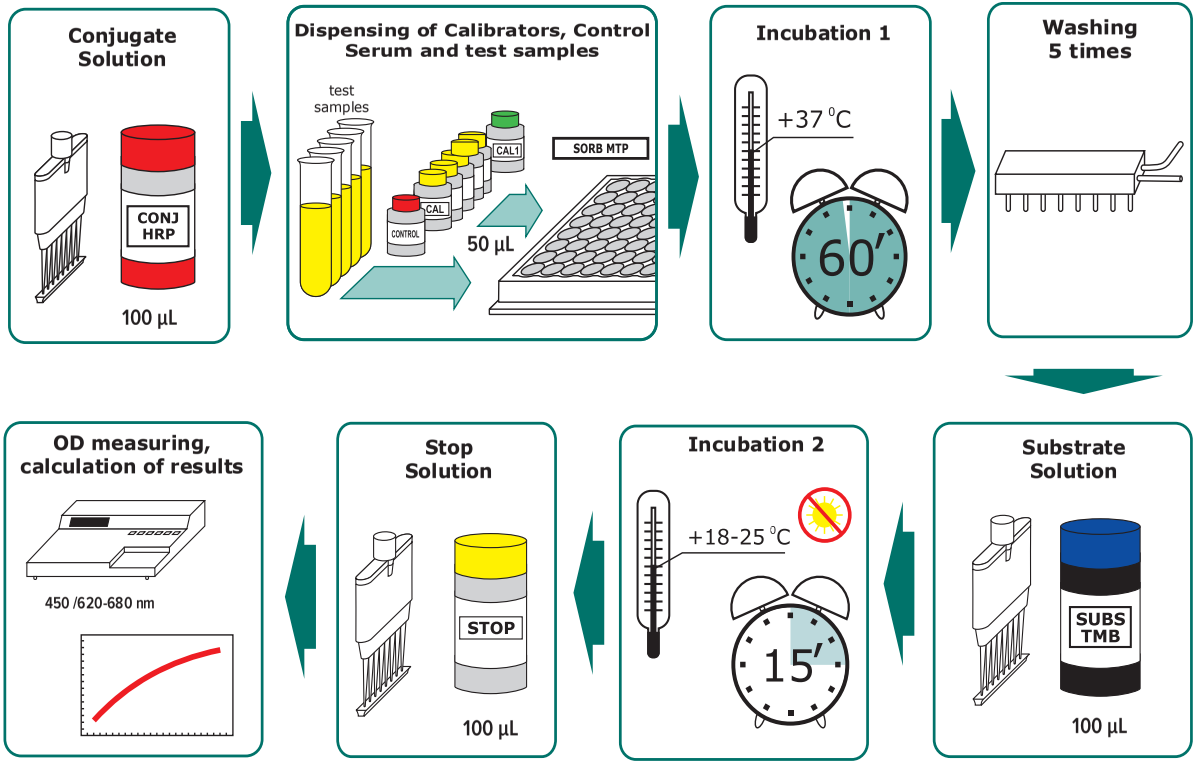
ASSAY PROCEDURE



K225

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of alpha-fetoprotein in human serum or plasma
AFP EIA

1. INTENDED USE

The AFP EIA kit is an enzyme immunoassay, intended for the quantitative determination of alpha-fetoprotein in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Alpha-fetoprotein (AFP) is a glycoprotein with a MW ca. 65 kDa which is secreted by fetal liver and yolk sac. AFP represents the main protein of fetal serum while being found in trace quantities in adults. Serum AFP quantitative determination is used in primary diagnostics and monitoring of hepatocellular liver cancer, trophoblastic tumours of testicles and ovary as well as theratomas and theratocarcinomas.

Quantitative determination of AFP in serum of pregnant women or in amniotic fluid during week 15-20 of gestation is widely used for laboratory screening of Down syndrome and defects of spinal cord.

3. PRINCIPLE OF THE TEST

The determination of the alpha-fetoprotein (AFP) is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to human AFP. Second antibodies – murine monoclonal antibodies to human AFP conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage AFP from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized AFP;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3’,5,5’-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured AFP in the serum specimen (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of AFP in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P225Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to human AFP; ready to use
C225Z	CAL 1	**Calibrator C1**	6 mL	1	Solution based on tris buffer (pH 7.2-7.4), free of human AFP, with preservative, ready to use (yellow liquid)
C225Z	CAL 2-6	**Calibrators**	0.8 mL	5	Solution based on tris buffer (pH 7.2-7.4), containing 5; 15; 50, 150 and 500 IU/mL of AFP, with preservative, ready to use (red liquids)
Q225Z	CONTROL	**Control Serum**	0.8 mL	1	Solution based on human serum, containing of known AFP content, with preservative, ready to use (colourless liquid)
T225Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of murine monocnoclonal antibodies to human AFP conjugated to the horseradish peroxidase; ready to use (red liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength;
– dry thermostat for +37°C±2°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLE

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The AFP EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The AFP EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;

  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 500 IU/mL, additionally dilute this sample accordingly, using (Calibrator C1). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of the blood serum (plasma) sample

Do not dilute Control Serum and Calibrators!

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 14 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-6) and 2 wells for Control Serum (Q)).

10.2 If necessary, dilute the test samples as described in 9.4.

10.3 Dispense **100 µL of Conjugate Solution** to all wells.

10.4 Dispense **50 µL of Calibrators and Control Serum as well as 50 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5								
E	CAL5	CAL5	SAMP6	SAMP6								
F	CAL6	CAL6	SAMP7	SAMP7								
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.5 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.6 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the wash solution volume can be increased to 350 µL.

10.7 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.8 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.9 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the Stop Solution. Set photometer blank on CAL1.

10.10 Plot a calibration curve in linear coordinates: (x) is the AFP concentration in the calibrators IU/mL, (y) – OD versus AFP concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve

10.11 Determine the corresponding concentration of AFP in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

12.1. Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for AFP. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

NOTE: values of AFP concentrations in the tested samples that are below the LoD (0.9 IU/mL) and also exceed the value of the upper Calibrator (500 IU/mL) should be provided in the following form : «the AFP concentration of tested sample X is «lower than 0.9 IU/mL» or «higher than 500IU/mL».

12.2. The calibrators concentration values of the AFP EIA kit are expressed in IU/mL. To calculate concentrations in ng/mL, the received concentration value in IU/mL shall be multiplied by 1.25.

1 IU/mL = 1,25 ng/mL

Sex, age	Units, IU/mL		Units alternative, ng/mL	
	Lower limit	Upper limit	Lower limit	Upper limit
Healthy donors	-	10.0	-	12.5

Medians and SKO (recommended normal range 0.5-2.0)

Pregnancy, week	Median, IU/mL	SKO
14	21.7	0.43
15	28.3	0.47
16	30.0	0.51
17	36.3	0.49
18	43.8	0.52
19	53.3	0.50
20	60.0	0.55
21	63.3	0.57

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, IU/mL	CV, %
1	54.16	1.83
2	289	7.67

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, IU/mL	CV, %
1	55.34	5.75
2	288.47	2.63

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, IU/mL	Concentration2, IU/mL	Concentration3, IU/mL	CV, %
1	54.6	55.89	54.89	2.95
2	289.4	281.75	283.46	1.41

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the mezhurand. The bias was calculated for each sample and it was determined whether it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known AFP concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 5-500 IU/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest AFP concentration in the serum or plasma sample that is detected by the AFP EIA kit is no lower than 0.9 IU/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for AFP EIA kit is 5.0 IU/mL.

13.1.5 Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 12000 IU/mL.

13.1.6 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of AFP with other analytes is shown in the table:

Analyte	Cross-reactivity, %
Albumin	< 0.1
hCG	< 0.1
Lactogen	< 0.1

14. REFERENCES

1. Johnson, P.J, (2002) Tumor Markers in Primary Malignancies of the liver. In «Tumor Markers: Physiology, pathobiology, technology and clinical applications», ed. Dimandis E.P. AACC Press, Washingon pp 269-276.
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3. Christiansen, M. et al. Alpha-fetoprotein in plasma and serum of healthy adults: preanalytical, analytical and biological sources of variation and construction of age-dependent reference intervals. Scand J Invest 2001 61: 205-216
4. Trape, J. et al. Reference change value for a-Fetoprotein and its application in early detection of hepatocellular carcinoma in patients with hepatic disease. Clin Chem 2003 49(7): 1209-1211
5. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
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SAMPLES IDENTIFICATION PLAN

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LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

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Instruction for use
A solid-phase enzyme immunoassay kit
for the quantitative determination of
CA 15-3 (M12) in human serum or plasma

CA 15-3 (M12) EIA

Catalogue number REF **K226**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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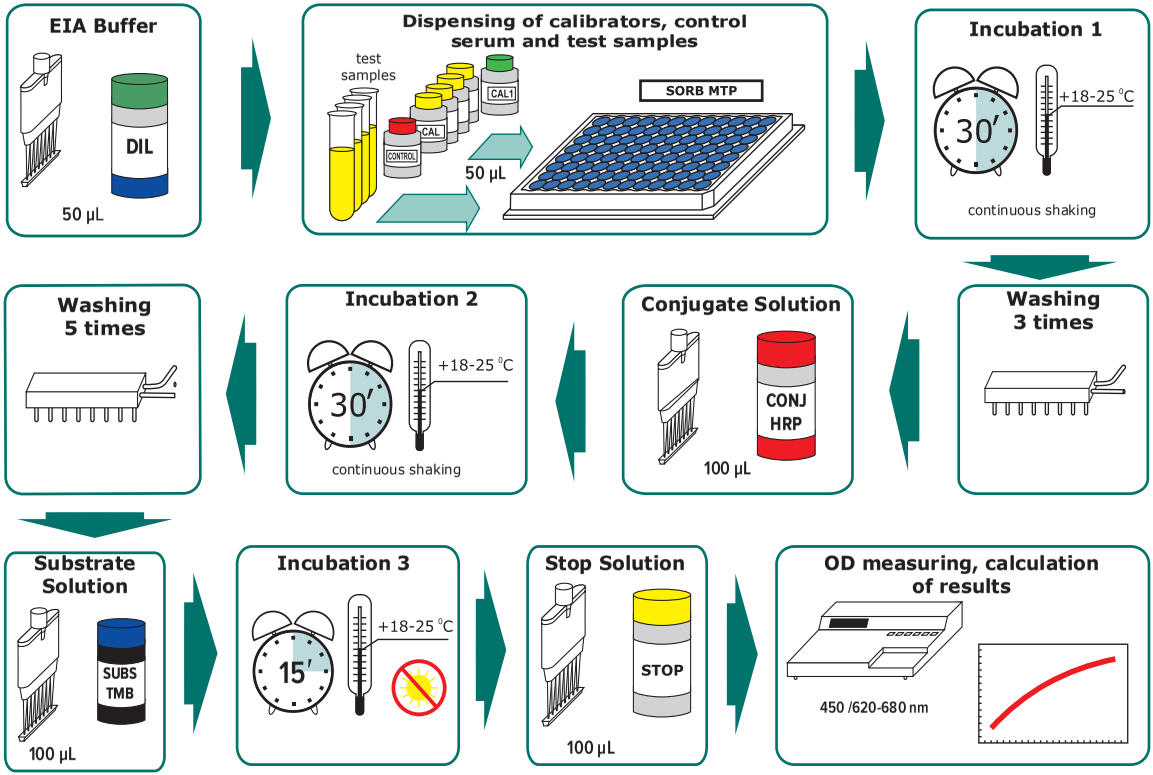
ASSAY PROCEDURE



K226

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of CA 15-3 (M12) in human serum or plasma
CA 15-3 (M12) EIA

1. INTENDED USE

The CA 15-3 (M12) EIA kit is an enzyme immunoassay, intended for the quantitative determination of CA 15-3 (M12) concentration in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

CA 15-3 or MUC1 is a heterogenous glycoprotein with a molecular mass ca. 300-450 kD. Elevation of serum CA 15-3 is associated with mammary carcinomas.

Quantitative determination of CA 15-3 in serum and plasma is helpful in monitoring of patients with such tumours to estimate the course of the disease, effectiveness of its treatment and to reveal recurrence or metastases. However, CA 15-3 values obtained should always be interpreted in context of results obtained by other diagnostic and clinical procedures. Besides mammary carcinomas, CA 15-3 levels in blood may rise in lung tumours, prostate cancer, ovarian carcinomas, gastro-intestinal tumours. Elevation of CA 15-3 level in blood can be also found in benign tumours of the mammary gland and the ovary, endometriosis, hepatitis, liver cirrhosis and lung fibrosis. Pregnancy and lactation may also cause elevation of CA 15-3 level in serum.

All CA 15-3 test systems are usually not very sensitive (not more than 75% even at stage III mammary carcinoma). Therefore, in monitoring of tumours, we recommend to use this test in conjunction with two other test kits designed by XEMA for diagnostics and monitoring of mammary carcinomas – M20 and M22. All three kits should be used to evaluate MUC1 concentration before and after surgery; the test kit showing the most pronounced postsurgery decline should be then used for further monitoring.

3. TEST PRINCIPLE

The determination of the CA 15-3 (M12) is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to human CA 15-3 (M12). Second antibodies – murine monoclonal antibodies to human CA 15-3 (M12) conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes three stages of incubation:

- during the first stage CA 15-3 (M12) from the specimen is captured by the antibodies coated onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized CA 15-3 (M12), fixed in the formed at the previous stage complexes;
- during the third stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured CA 15-3 (M12) in the serum specimen (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of CA 15-3 in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P226Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to human CA 15-3 (M12); ready to use
C226Z	CAL 1	**Calibrator C1**	0,6 mL	1	Solution based on phosphate buffer (pH 7.2-7.4), free of CA 15-3 (M12), with preservative, ready to use (colourless liquid)
C226Z	CAL 2-5	**Calibrators**	0,6 mL	4	Solutions based on phosphate buffer (pH 7.2-7.4), containing 12,5; 50; 125 and 250 U/mL CA 15-3 (M12), with preservative, ready to use (red liquids)
Q226Z	CONTROL	**Control Serum**	0,6 mL	1	Solution based on human serum, containing of known CA 15-3 (M12) content, with preservative, ready to use (colourless liquid)
T226Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of murine monoclonal antibodies to human CA 15-3 (M12) conjugated to the horseradish peroxidase; ready to use (red liquid)
S011Z	DIL	**EIA Buffer**	14 mL	1	Buffer solution with detergent and preservative, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	2	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (3 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm wavelength or 450\620-680 nm;
- shaker maintaining a speed of 600 - 800 rpm;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- vortex mixer;
- disposable gloves;
- absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The CA 15-3 (M12) EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The CA 15-3 (M12) EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- EIA Buffer, Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing solution preparation

Add the contents of the 22 mL washing solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the washing solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 250 U/mL, additionally dilute this sample accordingly, using EIA Buffer. Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of the blood serum (plasma) sample.

Do not dilute Control Serum and Calibrators!

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2 If necessary, dilute the test samples as described in 9.4

10.3 Dispense **50 µL of EIA Buffer** to all wells.

10.4 Dispense **50 µL of Calibrators and Control Serum as well as 50 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5								
D	CAL4	CAL4	SAMP6	SAMP6								
E	CAL5	CAL5	SAMP7	SAMP7								
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.5 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **30 minutes at room temperature (+18...+25°C) with continuous shaking 600-800 rpm.**

10.6 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 3 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.7 Add **100 µL of the Conjugate Solution** to all wells.

10.8 Cover strips with a plate sealing tape and incubate for **30 minutes at at room temperature (+18...+25°C) with continuous shaking 600-800 rpm.**

10.9 At the end of the incubation period, aspirate and wash each well 5 times as described in 10.6.

10.10 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.11 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.12 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the stop solution. Set photometer blank on CAL1.

10.13 Plot a calibration curve in linear coordinates: (x) is the concentration of CA 15-3 (M12) in the Calibrators U/mL, (y) – OD versus concentration of CA 15-3 (M12) (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.14 Determine the corresponding concentration of CA 15-3 (M12) in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for CA 15-3 (M12). Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered antimouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

NOTE: values of CA 15-3 (M12) concentrations in the tested samples that are below the LoD (0.75 U/mL) and also exceed the value of the upper calibrator (250 U/mL) should be provided in the following form : «the CA 15-3 (M12) concentration of tested sample X is «lower than 0.75 U/mL» or «higher than 250 U/mL»

Sex, age	Units, U/mL	
	Lower limit	Upper limit
Males	-	30
Females	-	30
Pregnancy:		
1st trimester	-	55
2nd trimester	5.0	65
3rd trimester	5.0	185
Lactation	-	120

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, U/mL	CV, %
1	23.5	6.23
2	150	3.17

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, U/mL	CV, %
1	23.47	4.25
2	148.44	7.63

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, U/mL	Concentration2, U/mL	Concentration3, U/mL	CV, %
1	25.6	26.17	28.49	5.72
2	153.4	157.5	159.45	1.97

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the mezhurand. The bias was calculated for each sample and it was determined whether it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known CA 15-3 (M12) concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 12.5-250 U/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest CA 15-3 (M12) concentration in the serum or plasma sample that is detected by the CA 15-3 (M12) EIA kit is no lower than 0.75 U/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for CA 15-3 (M12) EIA kit is 12.5 U/mL.

13.1.6 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of CA 15-3 (M12) with other analytes is shown in the table:

Analyte	Cross-reactivity, %
CEA	<0.1
CA 125	<0.1
CA 19-9	<0.1

14. REFERENCES

1. McGuire WL, Tandon AK, Allred D, Chamnes GC, Clark GM. How to use prognostic factors in axillary node negative breast cancer patients. J Natl Cancer Inst 1990; 82:1006-7.
2. Nicholson S, Richard J, Sainsbury C, et al. Epidermal growth factor receptor (EGFr): results of a 6-year follow up study in operable breast cancer with emphasis on the node-negative subgroup. Br J Cancer 1991; 63:146-50.
3. Somerville JE, Clarke LA, Biggart JD. C-erb B-2 overexpression and histological type of in-situ and invasive breast carcinoma. J Clin Pathol 1992;45-16-20.
4. Ueronese S, Gambacorta M. Detection of Ki-67 rate in breast cancer. AM J Clin Pathol 1991; 95:30-4.
5. Lotnicker M, Pavesi F, Scarabelli M. Tumor associated antigens CA15-3 and CA125 in ovarian cancer. Int. J. Biolog Markers 1991; 6:115
6. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
7. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
8. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

XEMA

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

qa@xema.com.ua

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of thyroglobulin in human serum or plasma

Thyroglobulin EIA

Catalogue number REF **K232**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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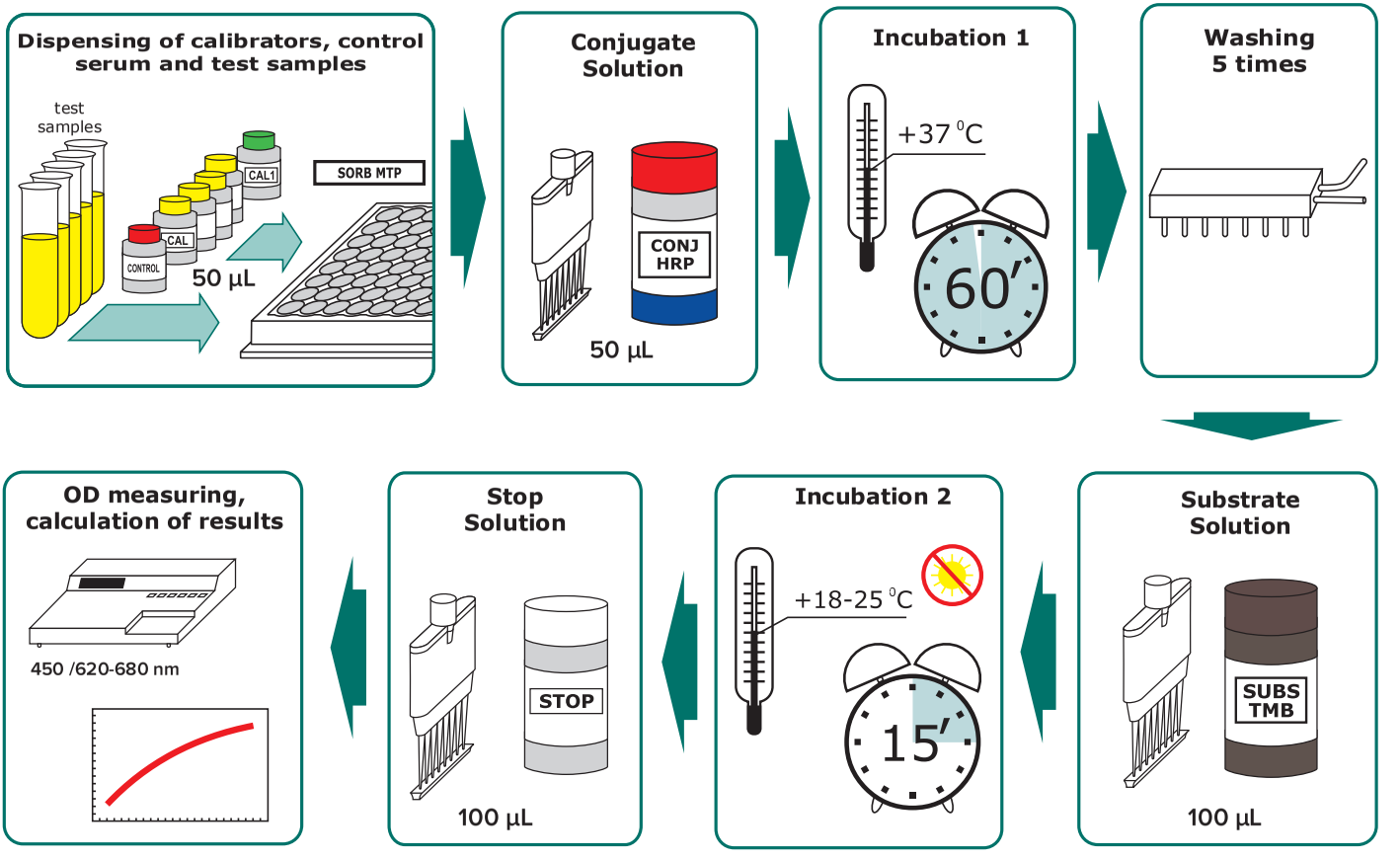
ASSAY PROCEDURE



During performing several independent series of tests, Calibrators and Control Serum should be used **each time**.

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of thyroglobulin in human serum or plasma

Thyroglobulin EIA

1. INTENDED USE

The Thyroglobulin EIA kit is an enzyme immunoassay, intended for the quantitative determination of thyroglobulin in human serum or plasma.

Quantitative determination of thyroglobulin in serum (plasma) is used to monitor patients with ovarian adenocarcinomas.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Thyroglobulin (TG) is a high molecular weight protein with a molecular weight of about 650-700 kDa, produced by the epithelial cells of the thyroid gland. In normal functioning of the thyroid gland, TG is produced in the lumen of the follicles and undergoes enzymatic iodination, which releases precursors of the hormones T3 and T4. Together with free hormones, small amounts of TS enter the circulation. The synthesis of TSH is regulated by the hormones of the pituitary-hypothalamic axis (thyroliberin and TSH), as well as by thyroid hormones themselves when administered for therapeutic purposes.

In patients with differentiated thyroid adenocarcinoma, serial determination of TG in serum (plasma) is a reliable monitoring marker of gland resection completeness, recurrence and metastatic growth of the tumour.

A significant increase in serum TSH levels is also observed in benign thyroid diseases. Thyroid diseases: thyroiditis and hyperthyroidism of various etiologies. In serial determination of TG can also be used for monitoring and prognosis of thyrostatic therapy of Graves' disease.

3. TEST PRINCIPLE

The determination of the TG is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to human TG. Second antibodies – murine monoclonal antibodies to human TG conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage TG from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized TG;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured TG in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density on the content of TG in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P232Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to human TG, ready to use
C232Z	CAL 1	**Calibrator C1**	0.6 mL	1	Solution based on tris buffer (pH 7.2-7.4), free of human TG, with preservative, ready to use (colourless liquid)
C232Z	CAL 2-5	**Calibrators**	0.6 mL	4	Solutions based on tris buffer (pH 7.2-7.4), containing 10; 25; 100 and 400 ng/mL of human TG, ready to use (red liquids)
Q232Z	CONTROL	**Control Serum**	0.6 mL	1	Solution based on human serum, containing of known human TG content, with preservative, ready to use (colourless liquid)
T232Z	CONJ HRP	**Conjugate Solution**	6 mL	1	Solution of murine monocnoclonal antibodies to TG conjugated to the horseradish peroxidase, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution, ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid, ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (1 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450/620-680 nm wavelength;
– dry thermostat for 37°C±1°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step, immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The Thyroglobulin EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The Thyroglobulin EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

10. ASSAY PROCEDURE

10.1. Put the desired number of strips into the frame based on the number of test samples and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2. Dispense **50 µL of Calibrators and Control Serum as well as 50 µL of test serum/ plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

During performing several independent series of tests, Calibrators and Control Serum should be used each time!

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5								
D	CAL4	CAL4	SAMP6	SAMP6								
E	CAL5	CAL5	SAMP7	SAMP7								
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.3. Add **50 µL of Conjugate Solution** to all wells.

10.4. Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.5. At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well **5 times** using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5 µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/ analyzer, the wash solution volume can be increased to 350 µL.

10.6. Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.
The incubation time can be varied depending on the intensity of the blue colour development.

10.7. Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.8. Read the optical density (OD) of the wells at 450 nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.9. Plot a calibration curve in linear coordinates: (x) is the TG concentration in the calibrators ng/mL, (y) – OD versus TG concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. For the algorithm calculation (approximation) of the calibration curve, using the interval (segment-linear, point-to-point) method is recommended.

10.10. Determine the corresponding concentration of TG in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is below 0.15, the OD of CAL5 is abowe the critical value (see Quality control Data Sheet) and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on the results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for TG. Based on data obtained by XEMA, the following normal range is recommended (see below).

NOTE: values of TG concentrations in the tested samples that are below the LoD (0.5 ng/mL) and also exceed the value of the upper Calibrator (400 ng/mL) should be provided in the following form: «the TG concentration of tested sample X is «lower than 0.5 ng/mL» or «higher than 400 ng/mL».

Sex, age	Одиниці, ng/mL	
	Lower limit	Upper limit
Healthy donors	-	50

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, ng/mL	CV, %
1	125.12	4.2
2	67.64	3.8

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, ng/mL	CV, %
1	126.27	3.0
2	67.87	7.4

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, ng/mL	Concentration1, ng/mL	Concentration1, ng/mL	CV, %
1	125.32	126.02	126.81	0.59
2	67.71	67.56	66.32	1.14

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the mezhurand. The bias was calculated for each sample and it was determined whether it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known TG concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 10–100 ng/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest TG concentration in the serum or plasma sample that is detected by the Thyroglobulin EIA kit is no lower than 0.5 ng/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for Thyroglobulin EIA kit is 10 ng/mL.

13.1.5 Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 400 ng/mL.

13.1.6 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

14. REFERENCES

1. CA Spencer, M Takeuchi, and M Kazarosyan – Current status and performance goals for serum thyroglobulin assays. Clin. Chem., Jan 1996; 42: 164 – 173.
2. PW Ladenson – Optimal laboratory testing for diagnosis and monitoring of thyroid nodules, goiter, and thyroid cancer. Clin. Chem., Jan 1996; 42: 183 – 187.
3. Anthony P. Weetman – Graves' Disease. N. Engl. J. Med., Oct 2000; 343: 1236 – 1248.
4. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
5. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
6. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81).

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

Instruction version/date: 2024.07

K232IE

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

Instruction version/date: 2024.07

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

qa@xema.com.ua

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of free β human chorionic gonadotropin in human serum or plasma

free β-HCG EIA

Catalogue number REF **K235**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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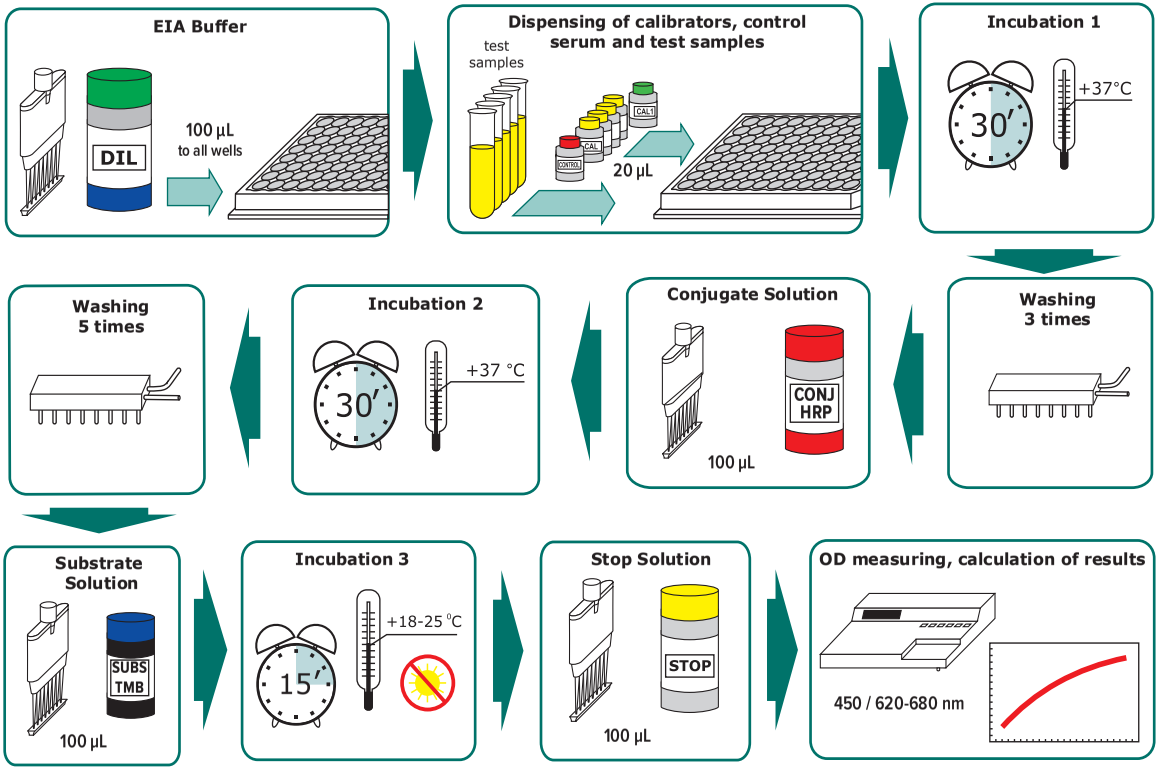
tel.:+ 49 911 931 639 67
E-mail: info@polmed.de
www.polmed.de

ASSAY PROCEDURE



K235

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of free β human chorionic gonadotropin in human serum or plasma
free β-HCG EIA

1. INTENDED USE

The free β-HCG EIA kit is an enzyme immunoassay, intended for the quantitative determination of free β human chorionic gonadotropin concentration in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Human chorionic gonadotropin (HCG) is a glycoprotein hormone secreted by trophoblastic cells of placenta during pregnancy. HCG appears in blood and urine in about 7-13 day after fertilization, reaching its maximum by the end of the first trimester. An intact molecule of HCG consists of two non-covalently bound polypeptide chains: α- and β-subunits. β-subunit is specific for HCG hormone while α-chain is identical in TSH, LH, FSH and HCG.

Normally, blood levels of free α- and β-chains reach not more than 0.5-1.0% of intact HCG level and during pregnancy vary in parallel with intact HCG. Recently, it was shown that, compared to control, a significant elevation of free β-chain is found in trisomy 21 (Down syndrome), the most pronounced difference being found during weeks 8-9 of pregnancy. That is why determination of free β-chain of HCG in conjunction with other markers (PABB-A, AFP) may be used to estimate risk of congenital pathology of the fetus.

In oncology, a marked rise of free β-chain in blood is found in trophoblastic and germinal tumours (choriocarcinoma, carcinoma of ovaries, etc.).

3. TEST PRINCIPLE

The determination of free β-HCG is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to human free β-HCG. Second antibodies – murine monoclonal antibodies to human free β-HCG conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes three stages of incubation:

- during the first stage free β-HCG from the specimen is captured by the antibodies coated onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized free β-HCG, fixed in the formed at the previous stage complexes;
- during the third stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured free β-HCG in the serum specimen (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of free β-HCG in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P235Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to human free β-HCG; ready to use
C235Z	CAL 1	**Calibrator C1**	0.8 mL	1	Solution based on tris buffer (pH 7.2-7.4), free of human free β-HCG, with preservative, ready to use (colourless liquid)
C235Z	CAL 2-5	**Calibrators**	0.8 mL	4	Solutions based on tris buffer (pH 7.2-7.4), containing 10; 50; 120 and 250 ng/mL of human free β-HCG, with preservative, ready to use (green liquids)
Q235Z	CONTROL	**Control Serum**	0.8 mL	1	Solution based on human serum, containing of known human free β-HCG content, with preservative, ready to use (colourless liquid)
T235Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of murine monoclonal antibodies to human free β-HCG conjugated to the horseradish peroxidase; ready to use (purple liquid)
S011Z2	DIL	**EIA Buffer**	22 mL	1	Buffer solution with detergent and preservative, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	2	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (3 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm wavelength or 450\620-680 nm;
- dry thermostat for +37 °C±2°C;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- vortex mixer;
- disposable gloves;
- absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The free β-HCG EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The free β-HCG EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- EIA Buffer, Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;

  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing solution preparation

Add the contents of the 22 mL washing solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the washing solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 250 ng/mL, additionally dilute this sample accordingly, using EIA Buffer. Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of the blood serum (plasma) sample.

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2 If necessary, dilute the test samples as described in 9.4

10.3 Dispense **100 µL of EIA Buffer** to all wells.

10.4 Dispense **20 µL of Calibrators and Control Serum as well as 20 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5								
D	CAL4	CAL4	SAMP6	SAMP6								
E	CAL5	CAL5	SAMP7	SAMP7								
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.5 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.6 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 3 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.7 Add **100 µL of the Conjugate Solution** to all wells.

10.8 Cover strips with a plate sealing tape and incubate for **30 minutes at +37°C.**

10.9 At the end of the incubation period, aspirate and wash each well 5 times as described in 10.6.

10.10 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.11 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.12 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the stop solution. Set photometer blank on CAL1.

10.13 Plot a calibration curve in linear coordinates: (x) is the concentration of free β-HCG in the Calibrators ng/mL, (y) – OD versus concentration of free β-HCG (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.14 Determine the corresponding concentration of free β-HCG in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

12.1. Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for free β-HCG. Based on data obtained by XEMA LLC, the following normal range is recommended (see below).

NOTE: values of free β-HCG concentrations in the tested samples that are below the LoD (1 ng/mL) and also exceed the value of the upper calibrator (250 ng/mL) should be provided in the following form : «the free β-HCG concentration of tested sample X is «lower than 1 ng/mL» or «higher than 250 ng/mL»

12.2. Expected values and references for the first trimester of pregnancy during calculating the risk of Down syndrome.

The medians below are based on using this kit during analyzing 2108 sera from pregnant women.

The values shown in the table are for guidance only and can be used to calculate the risk of Down syndrome only at the accumulation stage of own medians in each laboratory. The median values may differ depending on geographic areas due to racial and population characteristics.

Pregnancy, week	Median, ng/mL	SD
9	64.3	0.67
10	62	0.62
11	49.2	0.64
12	39.5	0.60
13	39	0.64

12.3. Expected values and references for the second trimester of pregnancy during calculating the risk of Down syndrome.

The data below are based on the analysis of 644 sera from pregnant women in the laboratory of XEMA LLC. Pregnancy dates were determined by the date of the last menstrual period and rounded to the nearest whole month.

The data in the table are for guidance only and are not intended to calculate the risk of Down syndrome.

Pregnancy, week	Units, ng/mL	
	Lower limit	Upper limit
14	21.8	31
15	20.3	28
16	13.3	23
17	11.1	19.9
18	9.9	19.4

Medians and SD (the recommended range of references is 0.5-2.0 MoM).

As new data on medians are accumulated depending on new data - please send your data to control@xema.com.ua.

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, ng/mL	CV, %
1	8	1.56
2	232	2.17

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, ng/mL	CV, %
1	8.17	7.33
2	232.7	2.45

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, ng/mL	Concentration2, ng/mL	Concentration3, ng/mL	CV, %
1	8.14	8.65	8.23	3.26
2	230.4	234.7	232.46	0.92

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the mezhurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known free β-HCG concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 10-250 ng/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest free β-HCG concentration in the serum or plasma sample that is detected by the free β-HCG EIA kit is no lower than 1 ng/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for free β-HCG EIA kit is 10 ng/mL.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of free β-HCG with other analytes is shown in the table:

Analyte	Cross-reactivity, %
LH	< 1
FSH	< 0.2
Prolactin	< 0.5

14. REFERENCES

1. Schaelike M, Kossakiewicz M, Kossakiewicz A, Schild RL Examination of a first-trimester Down syndrome screening concept on a mix of 11,107 high- and low-risk patients at a private center for prenatal medicine in Germany. // Ultrasound Obstet Gynecol. 2009 May;33(5):518-23.
2. Wortelboer EJ, Koster MP, Stoutenbeek P,Elvers LH, Loeber JG, Visser GH, Schielen PC. First-trimester Down syndrome screening performance in the Dutch population; how to achieve further improvementβ // Prenat Diagn. 2009 Mar 17. [Epub ahead of print].
3. Linskens IH, Levitus M, Frans A, Schielen PC, van Vugt JM, Blankenstein MA, Dijstelbloem HM. Performance of free β-human chorionic gonadotrophin (free β-hCG) and pregnancy associated plasma protein-A (PAPP-A) analysis between Delfia Xpress and AutoDelfia systems in The Netherlands. // Clin Chem Lab Med. 2009;47(2):222-6.
5. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
7. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
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LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
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LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

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Instruction for use
A solid-phase enzyme immunoassay kit
for the quantitative determination of
antigen CYFRA 21-1 in human serum or plasma

CYFRA 21-1 EIA

Catalogue number REF **K236**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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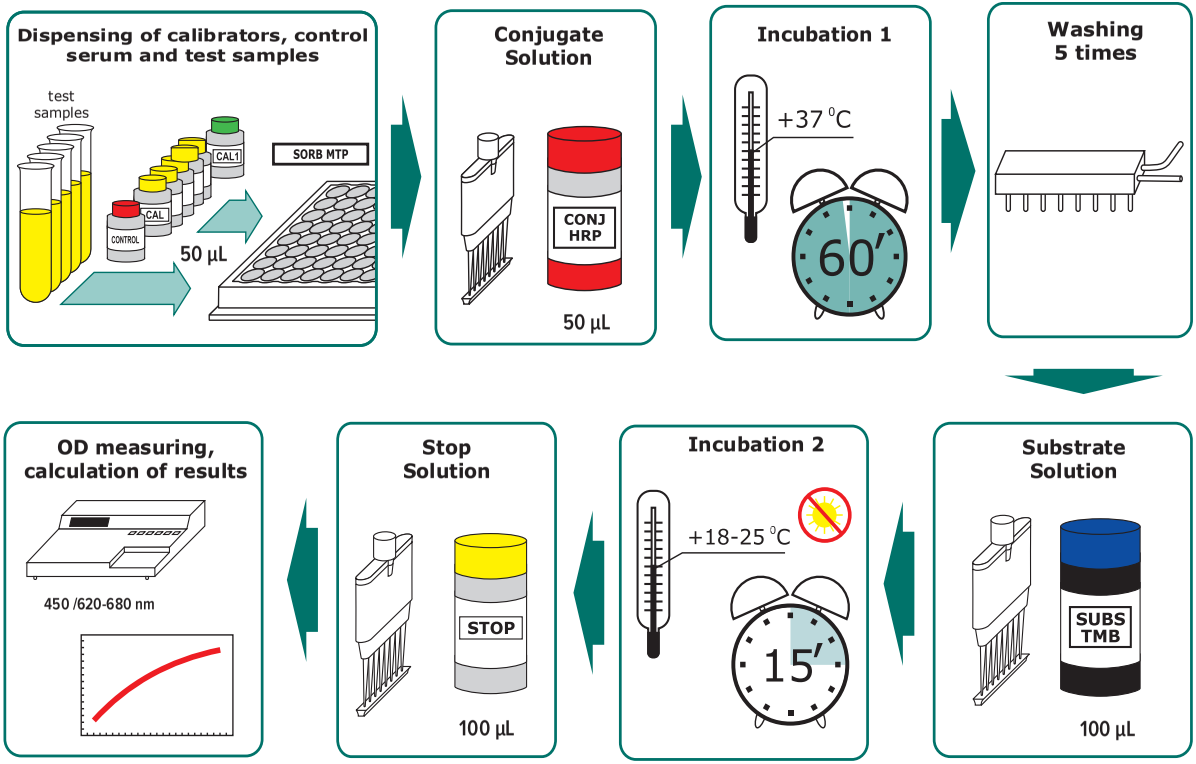
ASSAY PROCEDURE



K236

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of antigen CYFRA 21-1 in human serum or plasma
CYFRA 21-1 EIA

1. INTENDED USE

The CYFRA 21-1 EIA kit is an enzyme immunoassay, intended for the quantitative determination of antigen CYFRA 21-1 in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

The CYFRA 21-1 antigen is a fragment of cytokeratin 19, which is formed as a result of proteolysis and, unlike the main cytokeratin structure, is able to change into a soluble form and enter the systemic bloodstream.

The precursor molecule of the CYFRA 21-1 antigen - cytokeratin 19 - is expressed in all normal tissues, but a particularly high level of expression is observed in lung or bladder wall tumor cells.

Increased content of CYFRA 21-1 is observed in the blood of patients with lung tumors (mainly squamous cell carcinoma, less often adenocarcinoma and other histological forms) and bladder tumors. Determination of the level of the CYFRA 21-1 antigen is useful for monitoring the effectiveness of treatment and monitoring the course of these tumors; however, the results of the measurement of the CYFRA 21-1 antigen should always be interpreted in conjunction with the results of other research methods and clinical data.

3. TEST PRINCIPLE

The determination of the CYFRA 21-1 is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to soluble cytokeratin 8/19 (CYFRA 21-1). Second antibodies – murine monoclonal antibodies to human CYFRA 21-1 conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage CYFRA 21-1 from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized CYFRA 21-1;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured CYFRA 21-1in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density on the content of CYFRA 21-1in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P236Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to CYFRA 21-1; ready to use
C236Z	CAL 1	**Calibrator C1**	2 mL	1	Solution based on phosphate buffer (pH 7.2-7.4), free of CYFRA 21-1, with preservative, ready to use (colourless liquid)
C236Z	CAL 2-5	**Calibrators**	0.8 mL	4	Solution based on phosphate buffer (pH 7.2-7.4), containing 3; 10; 25 and 50 ng/mL of CYFRA 21-1, with preservative, ready to use (red liquids)
Q236Z	CONTROL	**Control Serum**	0.8 mL	1	Solution based on human serum, containing of known CYFRA 21-1 content, with preservative, ready to use (colourless liquid)
T236Z	CONJ HRP	**Conjugate Solution**	6 mL	1	Solution of murine monocnoclonal antibodies to CYFRA 21-1 conjugated to the horseradish peroxidase; ready to use (red liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength or 450\620-680 nm;
– dry thermostat for 37 °C±2 °C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The CYFRA 21-1 EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The CYFRA 21-1 EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 50 ng/mL, additionally dilute this sample accordingly, using (Calibrator C1). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of the blood serum (plasma) sample

Do not dilute Control Serum and Calibrators!

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2 If necessary, dilute the test samples as described in 9.4.

10.3 Dispense **50 µL of Calibrators and Control Serum as well as 50 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

10.4 Dispense **50 µL of Conjugate Solution** to all wells.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5								
D	CAL4	CAL4	SAMP6	SAMP6								
E	CAL5	CAL5	SAMP7	SAMP7								
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.5 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.6 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the wash solution volume can be increased to 350 µL.

10.7 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.8 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.9 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the Stop Solution. Set photometer blank on CAL1.

10.10 Plot a calibration curve in linear coordinates: (x) is the CYFRA 21-1 concentration in the calibrators ng/mL, (y) – OD versus CYFRA 21-1 concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.11 Determine the corresponding concentration of CYFRA 21-1 in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for CYFRA 21-1. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered antimouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

NOTE: values of CYFRA 21-1 concentrations in the tested samples that are below the LoD (0.5 ng/mL) and also exceed the value of the upper Calibrator (50 ng/mL) should be provided in the following form : «the CYFRA 21-1 concentration of tested sample X is «lower than 0.5 ng/mL» or «higher than 50 ng/mL».

Sex, age	Units, ng/mL	
	Lower limit	Upper limit
Healthy donors	-	3.0

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, ng/mL	CV, %
1	12.3	6.2
2	25	3.3

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, ng/mL	CV, %
1	12.27	4.3
2	25.89	5.2

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, ng/mL	Concentration2, ng/mL	Concentration3, ng/mL	CV, %
1	12.32	12.02	12.81	5.2
2	25.02	25.6	26.0	2.9

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the mezhurand. The bias was calculated for each sample and it was determined whether it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known CYFRA 21-1 concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 3-25 ng/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest CYFRA 21-1 concentration in the serum or plasma sample that is detected by the CYFRA 21-1 EIA kit is no lower than 0.5 ng/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for CYFRA 21-1 EIA kit is 3 ng/mL.

13.1.5 Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 50 ng/mL.

13.1.6 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of CYFRA 21-1 with other analytes is shown in the table:

Analyte	Cross-reactivity, %
CA 15-3	<0.1
CA 125	<0.1
CA 19-9	<0.1
AFP	<0.1
PSA	<0.1

14. REFERENCES

1. Petra Stieber CYFRA 21-1 (Cytokeratin-19-Fragment), in: Lothar Thomas, Labor und Diagnose, TH Brooks, Frankfurt, Germany
2. J-L Pujol, O Molinier, W Ebert et al. (2004) British Journal of Cancer 90 (11):2097-2105
3. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
4. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
5. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

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XEMA

SAMPLES IDENTIFICATION PLAN

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F												
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LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

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www.xema.in.ua

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of pregnancy-associated plasma protein A in human serum or plasma

PAPP-A EIA

Catalogue number REF **K238**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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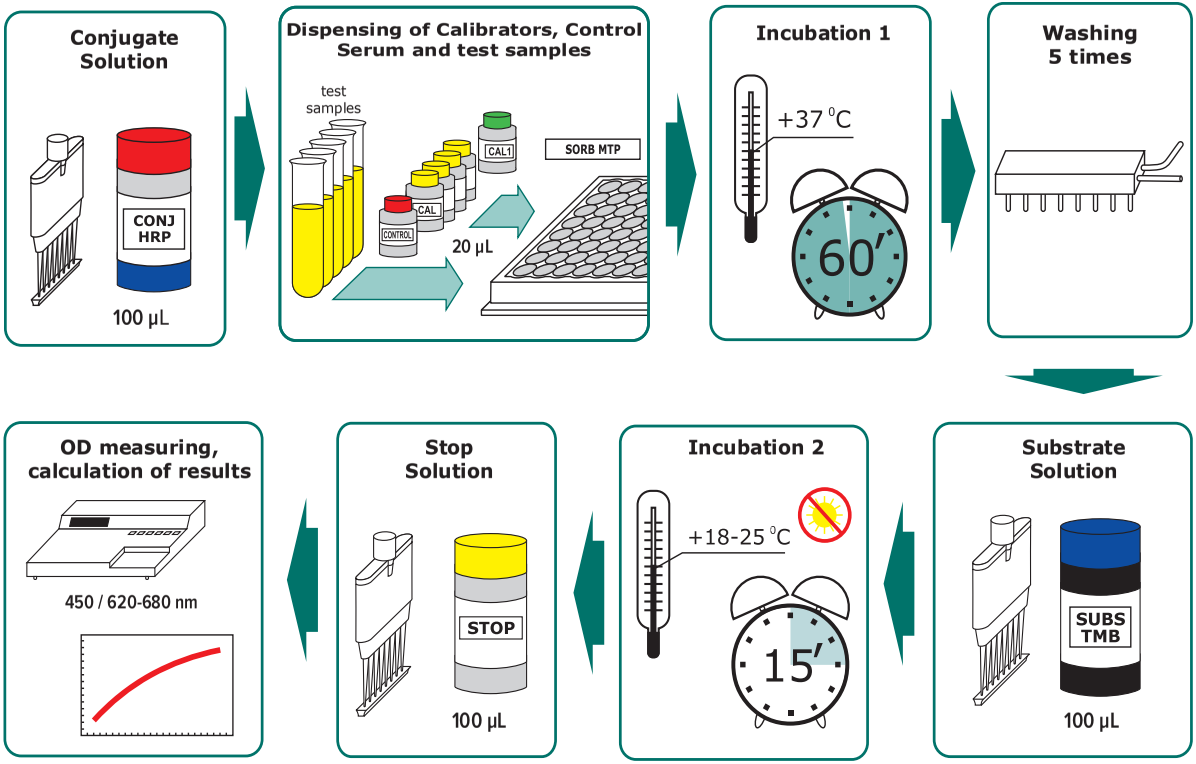
www.polmed.de

ASSAY PROCEDURE



K238

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of pregnancy-associated plasma protein A in human serum or plasma
PAPP-A EIA

1. INTENDED USE

The PAPP-A EIA kit is an enzyme immunoassay, intended for the quantitative determination of pregnancy-associated plasma protein A in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

PAPP-A (pregnancy-associated plasma protein A) is a high molecular weight glycoprotein consisting of two subunits. In normal pregnancy, PAPP-A level in maternal blood increases during the first two trimesters. Functional significance of PAPP-A during pregnancy remains unclear.

Lowered levels of PAPP-A are observed in Down's syndrome (trisomy 21) during weeks 8-12; after week 14, PAPP-A levels become similar to those in normal pregnancies. Low PAPP-A levels are also found in other trisomies (18 and 13) and chromosomal abnormalities in the fetus and in complicated pregnancies.

Determination of PAPP-A level in the first trimester is used in the following combinations of tests:

- PAPP-A + free beta-HCG.
- PAPP-A + free beta-HCG + USI of nuchal translucency.

In men and non-pregnant women, PAPP-A level is extremely low – usually, it is below the sensitivity level of most immunoassays. Recently, some evidence has appeared to confirm a link between raised PAPP-A levels and increased risk of complications in patients with coronary disease.

3. PRINCIPLE OF THE TEST

The determination of the pregnancy-associated plasma protein A (PAPP-A) is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to human PAPP-A. Second antibodies – murine monoclonal antibodies to human PAPP-A conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage PAPP-A from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized PAPP-A;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured PAPP-A in the serum specimen (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of PAPP-A in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P238Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to human PAPP-A; ready to use
C238Z	CAL 1	**Calibrator C1**	0.6 mL	1	Solution based on tris buffer (pH 7.2-7.4), free of human PAPP-A, with preservative, ready to use (colourless liquid)
C238Z	CAL 2-6	**Calibrators**	0.6 mL	5	Solution based on tris buffer (pH 7.2-7.4), containing 100; 500; 1000; 5000 and 10000 mU/L of PAPP-A, with preservative, ready to use (blue liquids)
Q238Z	CONTROL	**Control Serum**	0.6 mL	1	Solution based on human serum, containing of known PAPP-A content, with preservative, ready to use (colourless liquid)
T238Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of murine monocnoclonal antibodies to human PAPP-A conjugated to the horseradish peroxidase; ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength or 450\620-680 nm;
– dry thermostat for +37°C±2°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLE

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The PAPP-A EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The PAPP-A EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 14 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-6) and 2 wells for Control Serum (Q)).

10.2 Dispense **100 µL of Conjugate Solution** to all wells.

10.3 Dispense **20 µL of Calibrators and Control Serum as well as 20 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5								
E	CAL5	CAL5	SAMP6	SAMP6								
F	CAL6	CAL6	SAMP7	SAMP7								
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the wash solution volume can be increased to 350 µL.

10.6 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.7 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.8 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the Stop Solution. Set photometer blank on CAL1.

10.9 Plot a calibration curve in linear coordinates: (x) is the PAPP-A concentration in the calibrators mU/L, (y) – OD versus PAPP-A concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.10 Determine the corresponding concentration of PAPP-A in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

12.1. Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for PAPP-A. Based on data obtained by XEMA LLC, the following normal range is recommended (see below).

12.2. The medians below are based on the analysis of 1840 sera from pregnant women using this kit.

The values shown in the table are for guidance only and can be used to calculate the risk of Down syndrome only if each laboratory has accumulated its own medians. The median values may differ in different geographic areas due to racial and population characteristics.

NOTE: values of PAPP-AP concentrations in the tested samples that are below the LoD (10mU/L) and also exceed the value of the upper Calibrator (10000 mU/L) should be provided in the following form : «the PAPP-A concentration of tested sample X is «lower than 10 mU/L» or «higher than 10000 mU/L».

The calibrators concentration values of the PAPP-A EIA kit are expressed in mU/L. To calculate concentrations in µg/mL, the received concentration value in mU/L shall be multiplied by 0.0045.

1 mU/L = 0.0045 µg/mL

Sex, age	Units, mU/L		Units alternative, µg/mL	
	Lower limit	Upper limit	Lower limit	Upper limit
Males	-	150	-	0.68
Females	-	150	-	0.68

Medians and SD (the recommended range of references is 0.5-2.0 MoM).

Pregnancy, week	Median, mU/L	SD
9	969	2.9
10	1279	3.3
11	2153	3.4
12	3205	3.4
13	4250	3.6

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, mU/L	CV, %
1	2319	7.76
2	4879	7.08

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, mU/L	CV, %
1	5342	7.81
2	7853	7.92

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, mU/L	Concentration2, mU/L	Concentration3, mU/L	CV, %
1	4573	4765	4329	13.6
2	6634	6532	6791	8.1

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the mezhurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known PAPP-A concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 100-1000 mU/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest PAPP-A concentration in the serum or plasma sample that is detected by the PAPP-A EIA kit is no lower than 10 mU/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for PAPP-A EIA kit is 50mU/L.

13.1.5 Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 10000 mU/L.

13.1.6 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

14. REFERENCES

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3. Linskens IH, Levitus M, Frans A, Schielen PC, van Vugt JM, Blankenstein MA, Dijstelbloem HM. Performance of free beta-human chorionic gonadotrophin (free betahCG) and pregnancy associated plasma protein-A (PAPP-A) analysis between Delfia Xpress and AutoDelfia systems in The Netherlands. // Clin Chem Lab Med. 2009;47(2):222-6.
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6. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
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SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

qa@xema.com.ua

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of carbohydrate antigen 242 in human serum or plasma

CA 242 EIA

Catalogue number REF **K243**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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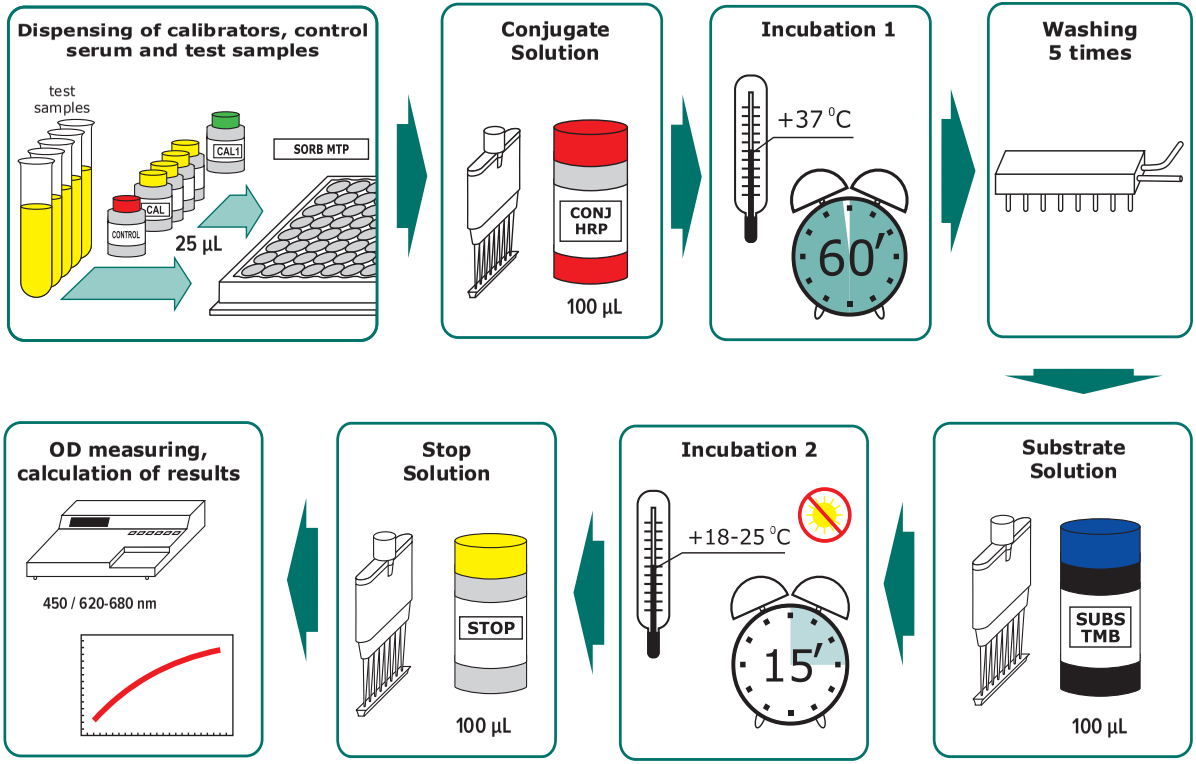
www.polmed.de

ASSAY PROCEDURE



K243

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of carbohydrate antigen 242 in human serum or plasma
CA 242 EIA

1. INTENDED USE

The CA 242 EIA kit is an enzyme immunoassay, intended for the quantitative determination of carbohydrate antigen 242 in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

The carbohydtrate antigen CA 242 is one of the most advanced markers of gastrointestinal cancer. CA 242 is found on cells of colonal mucosa as well as on apical part of cells lining pancreatic ducts.

CA 242 is one of the most important markers used in oncology. For differential diagnostics between pancreatic cancer (PC) and chronic pancreatitis, diagnostic specificity of CA 242 is 1.4 fold higher than that of CA 19-9. In patients with PC, a positive prognostic value of CA 242 determination is higher than that of CA 19-9 at any stage of the disease.

3. TEST PRINCIPLE

The determination of the CA 242 is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to CA 242/CA 19-9. Second antibodies – murine monoclonal antibodies to human CA 242 conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage CA 242 from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized CA 242;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3’,5,5’-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured CA 242 in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density on the content of CA 242 in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P223Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to CA 242/CA 19-9; ready to use
C243Z	CAL 1	**Calibrator C1**	2 mL	1	Solution based on tris buffer (pH 7.2-7.4), free of CA 242, with preservative, ready to use (colourless liquid)
C243Z	CAL 2-5	**Calibrators**	0.5 mL	4	Solutions based on tris buffer (pH 7.2-7.4), containing 15; 50; 100 and 200 U/mL of CA 242, with preservative, ready to use (blue liquids)
Q243Z	CONTROL	**Control Serum**	0.5 mL	1	Solution based on human serum, containing of known CA 242 content, with preservative, ready to use (colourless liquid)
T243Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of murine monoclonal antibodies to human CA 242 conjugated to the horseradish peroxidase; ready to use (red liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

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– microplate photometer with 450 nm wavelength or 450\620-680 nm;
– dry thermostat for +37°C±2°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
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ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

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Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

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Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The CA 242 EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The CA 242 EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;

  NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 200 U/mL, additionally dilute this sample accordingly, using (Calibrator C1). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of the blood serum (plasma) sample

10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2 If necessary, dilute the test samples as described in 9.4.

10.3 Dispense **25 µL of Calibrators and Control Serum as well as 25 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5								
D	CAL4	CAL4	SAMP6	SAMP6								
E	CAL5	CAL5	SAMP7	SAMP7								
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.4 Dispense **100 µL of Conjugate Solution** to all wells.

10.5 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.6 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the wash solution volume can be increased to 350 µL.

10.9 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.10 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.11 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the Stop Solution. Set photometer blank on CAL1.

10.13 Plot a calibration curve in linear coordinates: (x) is the CA 242 concentration in the calibrators U/mL, (y) – OD versus CA 242 concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.14 Determine the corresponding concentration of CA 242 in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

12.1. Therapeutical consequences should not be based on results of IVD methods alone - all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for CA 242. Based on data obtained by XEMA LLC, the following normal range is recommended (see below).

NOTE: values of CA 242 concentrations in the tested samples that are below the LoD (0.5 U/mL) and also exceed the value of the upper Calibrator (200 U/mL) should be provided in the following form : «the CA 242 concentration of tested sample X is «lower than 0.5 U/mL» or «higher than 200 U/mL».

Sex, age	Units, U/mL	
	Lower limit	Upper limit
Males	-	20
Females	-	20

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, U/mL	CV, %
1	10.12	3.2
2	53.64	2.8

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, U/mL	CV, %
1	10.27	7.0
2	53.87	6.1

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration1, U/mL	Concentration2, U/mL	Concentration3, U/mL	CV, %
1	10.32	10.02	10.81	3.8
2	53.71	53.56	54.32	0.6

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the mezhurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known CA 242 concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 15-100 U/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest CA 242 concentration in the serum or plasma sample that is detected by the CA 242 EIA kit is no lower than 0.5 U/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for CA 242 EIA kit is 15 U/mL.

13.1.5 Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 200 U/mL.

13.1.6 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of CA 242 with other analytes is shown in the table:

Analyte	Cross-reactivity, %
CEA	<0.1
CA 15-3	<0.1

14. REFERENCES

1. Rana S, Dutta U, Kochhar R, Rana SV, Gupta R, Pal R, Jain K, Srinivasan R, Nagi B, Nain CK, Singh K. Evaluation of CA 242 as a tumor marker in gallbladder cancer. J Gastrointest Cancer. 2012 Jun;43(2):267-71.
2. Tian SB, Yu JC, Kang WM, Ma ZQ, Ye X, Cao ZJ, Yan C. Combined detection of CEA, CA 19-9, CA 242 and CA 50 in the diagnosis and prognosis of resectable gastric cancer. Asian Pac J Cancer Prev. 2014;15(15):6295-300.
3. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
4. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
5. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

qa@xema.com.ua

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of carbohydrate antigen 72-4 in human serum or plasma

CA 72-4 EIA

Catalogue number REF **K244**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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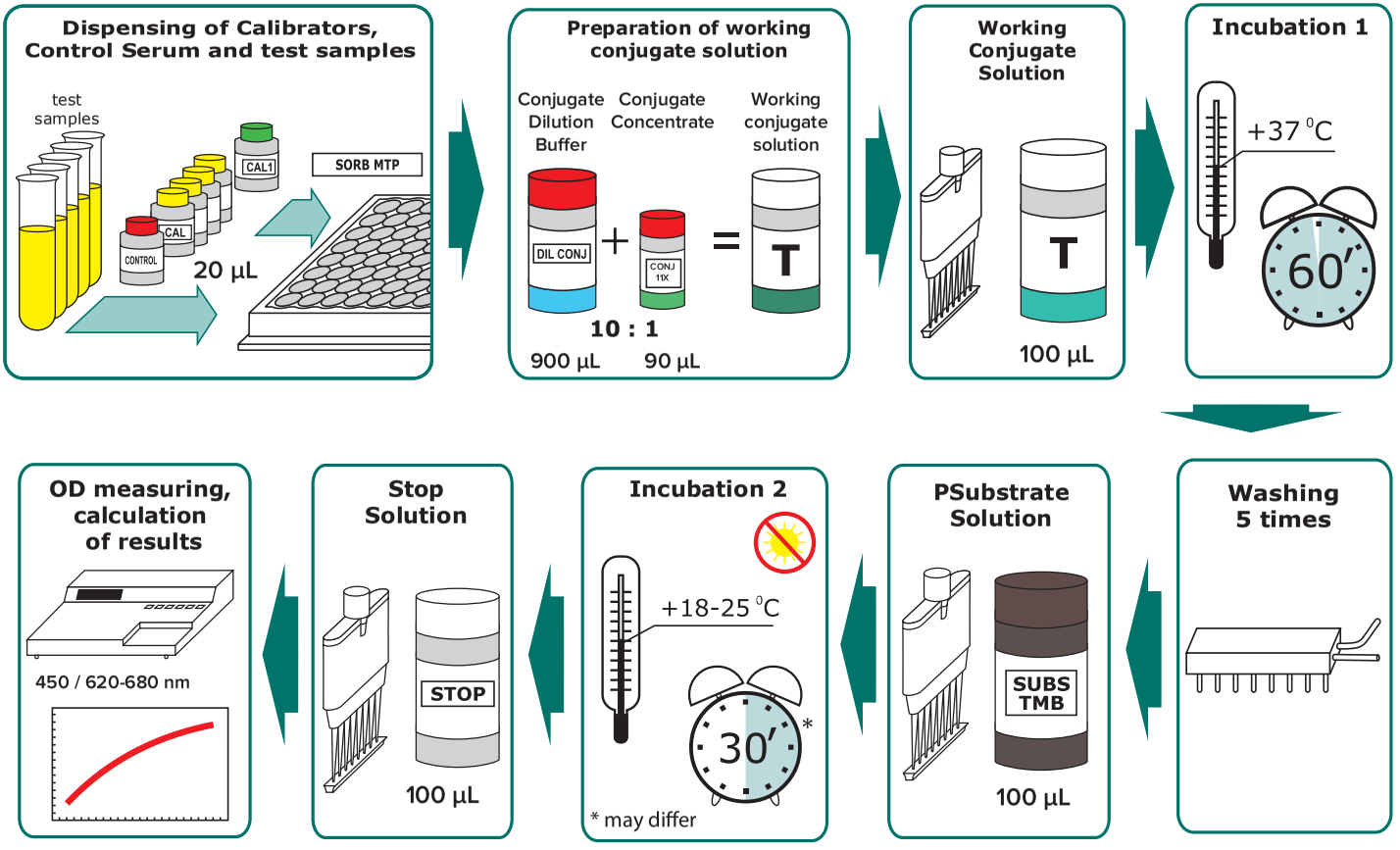
ASSAY PROCEDURE



During performing several independent series of tests, Calibrators, and Control Serum should be used each time.

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of carbohydrate antigen 72-4 in human serum or plasma
CA 72-4 EIA

1. INTENDED USE

The CA 72-4 EIA kit is an enzyme immunoassay, intended for the quantitative determination of carbohydrate antigen 72-4 in human serum or plasma.

Determination of CA 72-4 antigen concentration in serum (plasma) is used as an auxiliary method of early diagnosis, monitoring the effectiveness of therapy in malignant tumors of glandular tissue, such as gastric carcinoma, colon or ovarian cancer, for all population groups.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

CA 72-4, or a carbohydrate antigen 72-4, is a high MM (230-1000 kD) antigen (epitope) associated to gastric and ovarian cancer as well as some other malignancies and not expressed in noticeable quantities in tissues of healthy adult individuals.

Quantitative determination of CA 72-4 in serum or plasma is helpful (particularly, in combination with CA 19-9 – see XEMA LLC, Cat.# K223) for monitoring of gastric cancer and its therapy, while combined determination of CA 72-4 and CA 125 (see XEMA LLC, Cat.# K222) is used for monitoring of ovarian cancer.

Elevated levels of CA 72-4 are often seen in adenocarcinomas of the gastro-intestinal tract, ovaries (mucinous type) and lungs. Besides, raised CA 72-4 is sometimes also seen in patients with benign pathology (chronic inflammation, cysts, fibrosis). That is why, results of CA 72-4 determination should always be interpreted in conjunction with other laboratory and clinical data.

Functional purpose. Determination of the concentration of CA 72-4 antigen in serum (plasma) is used as an auxiliary method for early diagnosis, monitoring the effectiveness of therapy for malignant glandular tumours, such as gastric carcinoma, colon or ovarian cancer, for all population groups.

3. TEST PRINCIPLE

The determination of carbohydrate antigen 72-4 is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to human CA 72-4. Second antibodies – murine monoclonal antibodies to human CA 72-4 conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage CA 72-4 from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized CA 72-4;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3’,5,5’-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured CA 72-4 in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density on the content of CA 72-4 in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P244Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to human CA 72-4, ready to use
C244Z	CAL 1	**Calibrator C1**	0.5 mL	1	Solution based on human serum, free of CA 72-4, with preservative, ready to use (yellow liquid)
C244Z	CAL 2-5	**Calibrators**	0.5 mL	4	Solutions based on human serum, containing 5; 15; 50 and 200 U/mL of CA 72-4, with preservative, ready to use (blue liquids)
Q244Z	CONTROL	**Control Serum**	0.5 mL	1	Solution based on human serum, containing of known CA 72-4 content, with preservative, ready to use (yellow liquid)
T244XZ	CONJ 11X	**Conjugate Concentrate**	1.2 mL	1	Solution of murine monocnoclonal antibodies to human CA 72-4 conjugated to the horseradish peroxidase, 11x concentrate (green liquid)
ST244Z	DIL CONJ	**Conjugate Dilution Buffer**	12 mL	1	Buffer solution with detergent, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution, ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid, ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (1 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450\620-680 nm wavelength;
- dry thermostat for 37°C±1°C;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- vortex mixer;
- disposable gloves;
- absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The CA 72-4 EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The CA 72-4 EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Concentrate, Conjugate Dilution Buffer, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples should be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

9.4. Working conjugate solution preparation

Prepare in a different container a working conjugate solution by 11 dilutions of Conjugate Concentrate in Conjugate Dilution Buffer (eg, 90 µL of concentrate + 900 µL of Conjugate Dilution Buffer). In the case of partial use of the kit, take the necessary amount of Conjugate Concentrate and dilute it 11 times with Conjugate Dilution Buffer, since the working conjugate solution in a diluted form is not stored for a long time.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550
Volume of Conjugate Concentrate, mL	0.09	0.18	0.27	0.36	0.45	0.54	0.63	0.72	0.81	0.9	0.99	1.08
Volume of Conjugate Dilution Buffer, mL	0.9	1.8	2.7	3.6	4.5	5.4	6.3	7.2	8.1	9	9.9	10.8

10. ASSAY PROCEDURE

10.1. Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2. Dispense **20 µL of Calibrators and Control Serum as well as 20 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP3	SAMP3	SAMP11	SAMP11						
B	CAL2	CAL2	SAMP4	SAMP4	SAMP12	SAMP12						
C	CAL3	CAL3	SAMP5	SAMP5								
D	CAL4	CAL4	SAMP6	SAMP6								
E	CAL5	CAL5	SAMP7	SAMP7								
F	Q	Q	SAMP8	SAMP8								
G	SAMP1	SAMP1	SAMP9	SAMP9								
H	SAMP2	SAMP2	SAMP10	SAMP10								

10.3. Dispense **100 µL of Working conjugate solution** to all wells (see 9.4).

10.4. Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.5. At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well **5 times** using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6. Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 30 minutes.**
The incubation time can be varied depending on the intensity of the blue colour development.

10.7. Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.8. Read the optical density (OD) of the wells at 450 nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the stop solution. Set photometer blank on CAL1.

10.9. Plot a calibration curve in linear coordinates: (x) is the concentration of CA 72-4 in the Calibrators U/mL, (y) – OD versus concentration of CA 72-4 (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. For the algorithm calculation (approximation) of the calibration curve, using the interval (segment-linear, point-to-point) method is recommended.

10.10. Determine the corresponding concentration of CA 72-4 in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for CA 74-2. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered antimouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

NOTE: values of CA 74-2 concentrations in the tested samples that are below the LoD (0.3 U/mL) and also exceed the value of the upper calibrator (200 U/mL) should be provided in the following form : «the CA 74-2 concentration of tested sample X is «lower than 0.3 U/mL» or «higher than 200 U/mL».

Sex, age	Units, U/mL	
	Lower limit	Upper limit
Healthy donors	-	6.0

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1. Precision of Measurement

Reproducibility. The coefficient of variation of determining the content of CA 72-4 in the same sample of blood serum (plasma) using the kit CA 72-4 EIA does not exceed 10%.

13.1.2. Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the mezhurand. The bias was calculated for each sample and it was determined whether it corresponds to the specified limits of ± 10%.

13.1.3. Linearity

Linearity was determined using sera samples with known CA 72-4 concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 5–200 U/mL ±10%.

13.1.4. Analytical sensitivity

Limit of detection (LoD) – the lowest CA 72-4 concentration in the serum or plasma sample that is detected by the CA 72-4 EIA kit is no lower than 0.3 U/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for CA 72-4 EIA kit is 5 U/mL.

13.1.5. Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 20000 U/mL.

13.1.6. Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of CA 74-2 with other analytes is shown in the table:

Analyte	Cross-reactivity, %
CEA	<0.1
CA 125	<0.1
CA 19-9	<0.1

14. REFERENCES

1. DJ Byrne, MC Browning, and A Cuschieri – CA72-4: a new tumour marker for gastric cancer. Br J Surg, Sep 1990; 77(9): 1010-3.
2. Ian J. Jacobs and Usha Menon – Progress and Challenges in Screening for Early Detection of Ovarian Cancer. Mol. Cell. Proteomics, Apr 2004; 3: 355 – 366.
3. R Hamazoe, M Maeta, T Matsui, S Shibata, S Shiota, and N Kaibara – CA72-4 compared with carcinoembryonic antigen as a tumour marker for gastric cancer. Eur J Cancer, Jan 1992; 28A(8-9): 1351-4
4. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
5. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
6. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81).

SAMPLES IDENTIFICATION PLAN

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SAMPLES IDENTIFICATION PLAN

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LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

qa@xema.com.ua

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INSTRUCTION FOR USE
A SOLID-PHASE ENZYME IMMUNOASSAY KIT
FOR THE QUANTITATIVE DETERMINATION
OF C-PEPTIDE IN HUMAN SERUM OR PLASMA

C-peptide EIA

Catalogue number REF **K267C**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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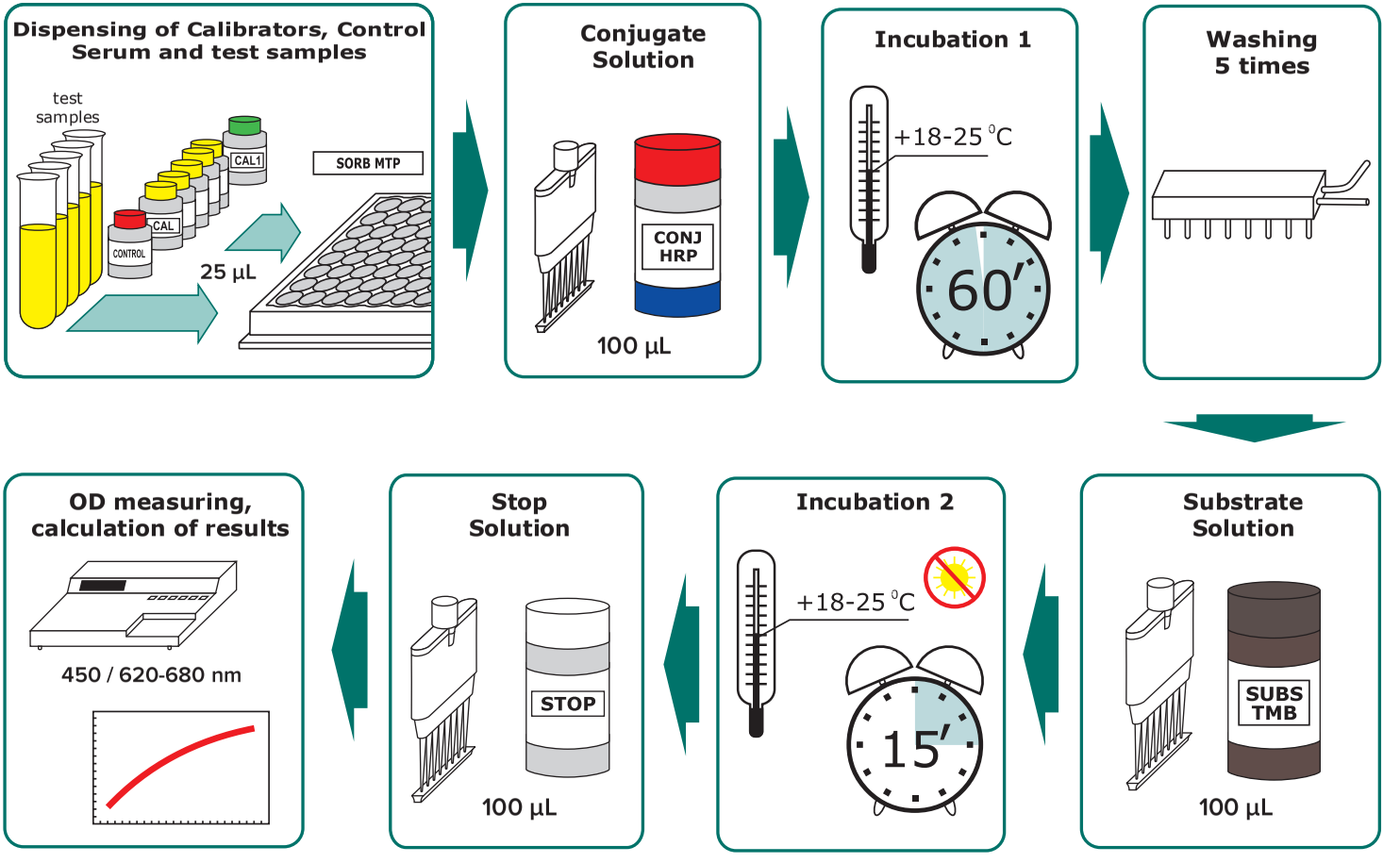
ASSAY PROCEDURE



K267C

CONTENT

INSTRUCTION FOR USE
A SOLID-PHASE ENZYME IMMUNOASSAY KIT
FOR THE QUANTITATIVE DETERMINATION OF C-PEPTIDE
IN HUMAN SERUM OR PLASMA

C-peptide EIA

1. INTENDED USE

A solid-phase enzyme immunoassay for the quantitative determination of C-peptide in blood serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

C-peptide is a component of the endocrine secretion of the pancreas. This protein is necessary for insulin synthesis in pancreatic cells, a multi-step process during which inactive proinsulin is broken down to release active insulin. C-peptide and insulin are secreted in equimolar amounts, so determining the level of C-peptide allows to estimate insulin secretion. It should be noted that although the number of C-peptide and insulin molecules formed during secretion into the bloodstream is the same, the molar concentration of C-peptide in the blood is approximately 5 times higher than the molar concentration of insulin, which is most likely due to the different rates of excretion of these substances from the bloodstream.

C-peptide determination has a number of advantages over insulin determination: the half-life of C-peptide in the bloodstream is longer than that of insulin, so the level of C-peptide is a more stable indicator than the concentration of insulin. In immunoassays, C-peptide does not cross-react with insulin, which makes it possible to measure C-peptide to assess insulin secretion even when taking exogenous insulin and in the presence of autoantibodies to insulin, which is important in the examination of patients with diabetes. The level of C-peptide changes in accordance with fluctuations in insulin levels. The ratio of these indicators may change in case of liver and kidney disease, as insulin is metabolised mainly by the liver, while C-peptide is metabolised and excreted by the kidneys. In this regard, the determination of this indicator can be useful for the correct interpretation of changes in blood insulin levels in case of liver function disorders.

3. TEST PRINCIPLE

The determination of C-peptide is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to human C-peptide. Second antibodies – murine monoclonal antibodies to human C-peptide conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage C-peptide from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized C-peptide;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3’,5,5’-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured C-peptide in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density on the content of C-peptide in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P267CZ	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to human C-peptide, ready to use
C267CZ	CAL 1	**Calibrator C1**	0.5 mL	1	Solution based on tris buffer (pH 7.2-7.4), free of human C-peptide, lyophilized
C267CZ	CAL 2-6	**Calibrators**	0.5 mL	5	Solutions based on tris buffer (pH 7.2-7.4), containing 0.3; 0.7; 3; 7 and 30 ng/mL of human C-peptide, contains blue dye, lyophilized Note. Concentrations of C-peptide in Calibrators may differ from the specified values, the exact values are indicated on the component labels
Q267CZ	CONTROL	**Control Serum**	0.5 mL	1	Solution based on human serum, containing of known C-peptide content, with preservative, lyophilized
T267CZ	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of murine monocnoclonal antibodies to human C-peptide conjugated to the horseradish peroxidase, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution, ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid, ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm and 620-680 nm wavelength;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- disposable gloves;
- absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step, immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The C-peptide EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The C-peptide EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- Calibrators and Control Serum after dissolving should be stored frozen in aliquots below **-15°C**.

NOTE: only one freezing/thawing cycle of Calibrators and Control Serum is allowed.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550

9.4.Calibrators and Control Serum preparation

Before first use of the kit dissolve the Calibrators and Control Serum: add 0.5 mL deionized water to each vial and mix thoroughly avoiding foam formation. Liquid Calibrators and Control Serum should be assayed within **72 hours**. For next assays Liquid Calibrators and Control Serum should be aliquoted and stored frozen below -15°C **immediately.**

10. ASSAY PROCEDURE

10.1. Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 14 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-6) and 2 wells for Control Serum (Q)).

10.2. Prepare Calibrators and Control Serum as described in 9.4.

10.3. Dispense **25 µL of Calibrators and Control Serum as well as 25 µL of test serum/ plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

NOTE: during performing several independent series of tests, Calibrators and Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CAL1	CAL1	SAMP2	SAMP2	SAMP10	SAMP10						
B	CAL2	CAL2	SAMP3	SAMP3	SAMP11	SAMP11						
C	CAL3	CAL3	SAMP4	SAMP4	SAMP12	SAMP12						
D	CAL4	CAL4	SAMP5	SAMP5								
E	CAL5	CAL5	SAMP6	SAMP6								
F	CAL6	CAL6	SAMP7	SAMP7								
G	Q	Q	SAMP8	SAMP8								
H	SAMP1	SAMP1	SAMP9	SAMP9								

10.4. Dispense **100 µL of Conjugate Solution** to all wells.

10.5. Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at room temperature (+18...+25°C)**.

10.6. At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well **5 times** using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5 µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the wash solution volume can be increased to 350 µL.

10.7. Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.8. Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.9. Read the optical density (OD) of the wells at 450 nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.10. Plot a calibration curve in linear coordinates: (x) is the C-peptide concentration in the calibrators ng/mL, (y) – OD versus C-peptide concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. For the algorithm calculation (approximation) of the calibration curve, using the interval (segment-linear, point-to-point) method is recommended.

10.11. Determine the corresponding concentration of C-peptide in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

Therapeutical consequences should not be based on the results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for C-peptide. Based on data obtained by XEMA LLC, the following normal range is recommended (see below).

NOTE: values of C-peptide concentrations in the tested samples that are below the LoD (0.015 ng/mL) and also exceed the value of the upper Calibrator (30 ng/mL) should be provided in the following form : «the C-peptide concentration of tested sample X is «lower than 0.015 ng/mL» or «higher than 30* ng/mL».*

* - concentration of the C-peptide of the upper calibration sample may slightly differ from the specified value, the exact value is indicated on the component label.

The calibrators concentration values of the C-peptide EIA kit are expressed in ng/mL. To calculate concentrations in pmol/L, the received concentration value in ng/mL shall be multiplied by 331.

1 ng/mL = 331 pmol/L

Sex, age	Units, ng/mL		Units alternative, pmol/L	
	Lower limit	Upper limit	Lower limit	Upper limit
Healthy donors	0.9	5.0	298	1655

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1. Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

Sample	Concentration, ng/mL	CV, %
1	5.12	3.2
2	3.32	2.8

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

Sample	Concentration, ng/mL	CV, %
1	5.27	4.0
2	3.87	2.4

Reproducibility between lots was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

Sample	Concentration 1, ng/mL	Concentration 2, ng/mL	Concentration 3, ng/mL	CV, %
1	5.32	5.02	5.81	7.2
2	3.71	3.56	3.32	6.6

13.1.2. Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3. Linearity

Linearity was determined using sera samples with known C-peptide concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 5-20 ng/mL ±10%.

13.1.4. Analytical sensitivity

Limit of detection (LoD) – the lowest C-peptide concentration in the serum or plasma sample that is detected by the C-peptide EIA kit is no lower than 0.015 ng/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for C-peptide EIA kit is 0.2 ng/mL.

13.1.5. Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 40 ng/mL.

13.1.6. Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

14. REFERENCES

1. Rui Lv, Yingshan Chen, Nana Xia, Yuxin Liang, Qiyi He, Minyou Li, Zongxian Qi, Yeyu Lu, Suqing Zhao, Development of a double-antibody sandwich ELISA for rapid detection to C-peptide in human urine, J Pharm Biomed Anal. 2019 Jan 5;162:179-184. doi: 10.1016/j.jpba.2018.07.049. Epub 2018 Aug 2.

2. Melanie L Graham, Sarah C Gresch, Sharon K Hardy, Lucas A Mutch, Jody L Janecek, Rebecca L Hegstad-Davies, Evaluation of commercial ELISA and RIA for measuring porcine C-peptide: implications for research, Xenotransplantation. 2015 Jan-Feb;22(1):62-9. doi: 10.1111/xen.12143. Epub 2014 Oct 7.

3. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».

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SAMPLES IDENTIFICATION PLAN

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A												
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F												
G												
H												

LOT _____ DATE _____

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

+38 044 294-69-78

or write to:

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Instruction for use

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ НАБОРА РЕАГЕНТОВ ДЛЯ ИММУНОФЕРМЕНТНОГО ОПРЕДЕЛЕНИЯ ОБЩЕГО IgG В БИОЛОГИЧЕСКИХ ЖИДКОСТЯХ

«общий IgG-ИФА»

A SOLID-PHASE ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF TOTAL IgG IN HUMAN BIOLOGICAL FLUIDS

Total IgG EIA

НОМЕР ПО КАТАЛОГУ REF **K271**

ТУ № 9398-271-18619450-2009

РЕГИСТРАЦИОННОЕ УДОСТОВЕРЕНИЕ
№ ФСР 2009/06104 от 19 ноября 2009 года

Антитела к ВИЧ 1,2, вирусу гепатита С и HBsAg отсутствуют
Контрольные сыворотки, входящие в состав набора, инактивированы.

For 96 determinations/На 96 определений

IVD Для *ин витро* диагностики

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Tel./fax: +7(495) 510-57-07
e-mail: redkin@xema-medica.com
internet: www.xema-medica.com

EC REP

Authorized Representative in EU:
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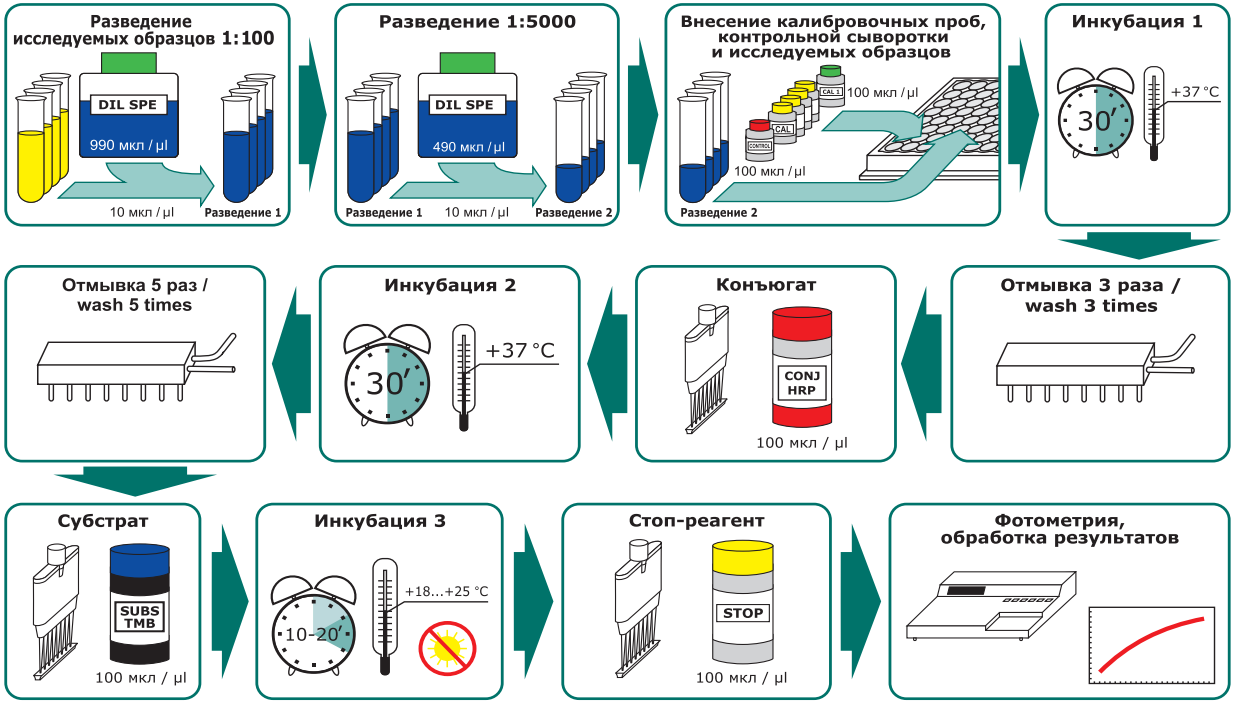
Схема проведения анализа / Test procedure *



K271

* Для сыворотки (плазмы) крови.
Способ разведения для других видов материала приведен в таблице М

* Blood serum or plasma
For other tested materials, see table M.

СОДЕРЖАНИЕ

CONTENT

Инструкция составлена Руководителем службы клиентского сервиса ООО «ХЕМА», к. б. н. Д. С. Кострикиным

«УТВЕРЖДЕНА»
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ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ НАБОРА РЕАГЕНТОВ ДЛЯ ИММУНОФЕРМЕНТНОГО ОПРЕДЕЛЕНИЯ ОБЩЕГО IgG В БИОЛОГИЧЕСКИХ ЖИДКОСТЯХ «общий IgG-ИФА»

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов «общий IgG-ИФА» предназначен для количественного определения концентрации общего IgG в биологических жидкостях (см. таблицу М) методом твердофазного иммуноферментного анализа.

1.2. Антитела класса IgG преобладают в составе фракции γ – глобулинов и являются основным классом антител, присутствующих в сыворотке крови человека. Увеличение концентрации IgG в сыворотке крови является основным признаком зрелого иммунного ответа, а снижение их концентрации ниже 5 г/л свидетельствует о развитии тяжелого иммунодефицита. Определение концентрации IgG в сыворотке крови, как и соотношения содержания IgG/IgA/IgM может служить одним из основных критериев оценки иммунного статуса индивида и использоваться при контроле лечения некоторых инфекционных заболеваний. Резкое повышение концентрации IgG в сыворотке крови наблюдается при миеломной болезни.

2. ПРИНЦИП РАБОТЫ НАБОРА

Определение общего IgG основано на использовании «сэндвич»-варианта твердофазного иммуноферментного анализа. На внутренней поверхности лунок планшета иммобилизованы мышиные моноклональные антитела к общему IgG человека. В лунках планшета, при добавлении исследуемого образца, происходит связывание общего IgG, содержащегося в исследуемом образце, с антителами на твердой фазе. Образовавшийся комплекс выявляют с помощью конъюгата мышиных моноклональных антител к общему IgG с пероксидазой хрена. В результате образуется связанный с пластиком «сэндвич», содержащий пероксидазу. Во время инкубации с раствором субстрата тетраметилбензидина (ТМБ) происходит окрашивание растворов в лунках. Интенсивность окраски прямо пропорциональна концентрации общего IgG в исследуемом образце. Концентрацию общего IgG в исследуемых образцах определяют по калибровочному графику зависимости оптической плотности от содержания общего IgG в калибровочных пробах.

3. АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Специфичность. Перекрестная реакция мышиных моноклональных антител к общему IgG с другими аналитами приведена в таблице:

Аналит	Перекрестная реакция, %
IgA	<0.1
IgM	<0.1
IgE	<0.1

3.2. Воспроизводимость.

Коэффициент вариации результатов определения содержания общего IgG в одном и том же образце биологических жидкостей с использованием Набора «общий IgG-ИФА» не превышает 8.0%.

3.3. Линейность.

Зависимость концентрации общего IgG в образцах биологических жидкостей при разведении их биологическими жидкостями, не содержащими общий IgG, имеет линейный характер в диапазоне концентраций 1–25 г/л и составляет ±10.0%.

3.4. Точность.

Данный аналитический параметр проверяется тестом на «открытие» – соответствие измеренной концентрации общего IgG предписанной, полученной путем смешивания равных объемов контрольной сыворотки и калибровочной пробы 5.0 г/л. Процент «открытия» составляет 90–110%.

3.5. Чувствительность.

Минимальная достоверно определяемая Набором «общий IgG-ИФА» концентрация общего IgG в биологических жидкостях не превышает 0.06 г/л.

4. СОСТАВ НАБОРА

	Код компонента	Символ	Наименование	Кол-во	Ед.	Описание
1	P271Z	SORB MTP	**Планшет** 96-луночный полистироловый, стрипированный, готов к использованию	1	шт.	-
2	C271Z	CAL 1–5	**Калибровочные пробы** на основе трис-буфера (pH 7.2-7.4), содержащие известные количества общего IgG – **0**; **1**; **5**; **10**; **25 г/л**, готовы к использованию (по 1 мл каждая)	5	шт.	прозрачные жидкости синего цвета (калибровочная проба 0 – прозрачная бесцветная жидкость)
3	Q271Z	CONTROL	**Контрольная сыворотка** на основе сыворотки крови человека с известным содержанием общего IgG, готова к использованию (1 мл)	1	шт.	прозрачная бесцветная жидкость
4	T271Z	CONJ HRP	**Конъюгат**, готов к использованию (14 мл)	1	шт.	прозрачная жидкость красного цвета
5	SP271Z	DIL SPE	**Буфер для разведения образцов,** готов к использованию (100 мл)	1	шт	прозрачная жидкость синего цвета
6	R055Z	SUBS TMB	**Раствор субстрата тетраметилбензидина** (ТМБ), готов к использованию (14 мл)	1	шт.	прозрачная бесцветная жидкость
7	S008Z	BUF WASH 26X	**Концентрат отмывочного раствора** (солевой раствор с твин-20 и бензойной кислотой), 26х-кратный (22 мл)	1	шт.	прозрачная бесцветная жидкость
8	R050Z	STOP	**Стоп-реагент**, готов к использованию (14 мл)	1	шт.	прозрачная бесцветная жидкость
9	N003	-	Бумага для заклеивания планшета	2	шт.	-
10	K271I	-	Инструкция по применению Набора реагентов «общий IgG-ИФА»	1	шт.	-
11	K271Q	-	Паспорт контроля качества Набора реагентов «общий IgG-ИФА»	1	шт.	-

5. МЕРЫ ПРЕДОСТОРОЖНОСТИ

5.1. Потенциальный риск применения Набора – класс 2а (ГОСТ Р 51609-2000).

5.2. Все компоненты Набора, за исключением стоп-реагента (5.0% раствор серной кислоты), в используемых концентрациях являются нетоксичными.

Раствор серной кислоты обладает раздражающим действием. Избегать разбрызгивания и попадания на кожу и слизистые. При попадании на кожу и слизистые пораженный участок следует промыть большим количеством проточной воды.

5.3. При работе с Набором следует соблюдать «Правила устройства, техники безопасности, производственной санитарии, противоэпидемического режима и личной гигиены при работе в лабораториях (отделениях, отделах) санитарно-эпидемиологических учреждений системы Министерства здравоохранения СССР» (Москва, 1981 г.).

5.4. При работе с Набором следует надевать одноразовые резиновые или пластиковые перчатки, так как образцы крови человека следует рассматривать как потенциально инфицированный материал, способный длительное время сохранять и передавать ВИЧ, вирус гепатита или любой другой возбудитель вирусной инфекции.

6. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ПРИ РАБОТЕ С НАБОРОМ

- фотометр вертикального сканирования, позволяющий измерять оптическую плотность содержимого лунок планшета при длине волны 450 нм;
- термостат, поддерживающий температуру +37 °С ±0.1 °С;
- дозаторы со сменными наконечниками, позволяющие отбирать объемы в диапазоне 10–250 мкл;
- цилиндр мерный вместимостью 1000 мл;
- вода дистиллированная;
- перчатки резиновые или пластиковые;
- бумага фильтровальная.

7. ПОДГОТОВКА РЕАГЕНТОВ ДЛЯ АНАЛИЗА

7.1. Перед проведением анализа компоненты Набора и исследуемые образцы сыворотки (плазмы) крови следует выдержать при комнатной температуре (+18...+25 °С) не менее 30 мин.

7.2. Приготовление планшета.

Вскрыть пакет с планшетом и установить на рамку необходимое количество стрипов. Оставшиеся неиспользованными стрипы, чтобы предотвратить воздействие на них влаги, тщательно заклеить бумагой для заклеивания планшета и хранить при температуре +2...+8 °С в течение всего срока годности Набора.

7.3. Приготовление отмывочного раствора.

Содержимое флакона с концентратом отмывочного раствора (22 мл), перенести в мерный цилиндр вместимостью 1000 мл, добавить 550 мл дистиллированной воды и тщательно перемешать. В случае дробного использования Набора следует отобрать необходимое количество концентрата отмывочного раствора и развести дистиллированной водой в 26 раз (1 мл концентрата отмывочного раствора + 25 мл дистиллированной воды).

8. УСЛОВИЯ ХРАНЕНИЯ И ЭКСПЛУАТАЦИИ НАБОРА

8.1. Набор реагентов «общий IgG-ИФА» должен храниться в упаковке предприятия-изготовителя при температуре +2...+8 °С в течение всего срока годности, указанного на упаковке Набора.

Допускается хранение (транспортировка) Набора при температуре до +25 °С не более 15 суток. Не допускается замораживание целого набора.

8.2. Набор рассчитан на проведение анализа в дубликатах 42 исследуемых образцов, 5 калибровочных проб и 1 пробы контрольной сыворотки (всего 96 определений).

8.3. В случае дробного использования Набора компоненты следует хранить следующим образом:

- оставшиеся неиспользованными стрипы необходимо тщательно заклеить бумагой для заклеивания планшета и хранить при температуре +2...+8 °С в течение всего срока годности Набора;
- Буфер для разведения образцов, конъюгат, субстрат, стоп-реагент после вскрытия флаконов следует хранить при температуре +2...+8 °С в течение всего срока годности Набора;
- калибровочные пробы и контрольную сыворотку после вскрытия флаконов следует хранить при температуре +2...+8 °С не более 2 месяцев;
- оставшийся неиспользованным концентрат отмывочного раствора следует хранить при температуре +2…+8 °С в течение всего срока годности Набора. Приготовленный отмывочный раствор следует хранить при комнатной температуре (+18…+25 °С) не более 15 суток или при температуре +2…+8 °С не более 45 суток.

Примечание. После использования реагента немедленно закрывайте крышку флакона. Закрывайте каждый флакон своей крышкой.

8.4. Для проведения анализа не следует использовать гемолизированную, мутную сыворотку (плазму) крови, а также сыворотку (плазму) крови, содержащую азид натрия. Если анализ производится не в день взятия крови, сыворотку (плазму) следует хранить при температуре -20 °С. Повторное замораживание-оттаивание образцов сыворотки (плазмы) крови не допускается. Допускается исследование сывороток, хранение которых с момента забора крови осуществлялось при температуре от +2 ºС до +8 ºС не более 7 суток.

8.5. Исключается использование для анализа образцов сыворотки (плазмы) крови людей, получавших в целях диагностики или терапии препараты, в состав которых входят мышиные антитела.

8.6. При использовании Набора для проведения нескольких независимых серий анализов следует иметь в виду, что для каждого независимого определения необходимо построение нового калибровочного графика; кроме этого, рекомендуется определение концентрации общего IgG в контрольной сыворотке.

8.7. Для получения надежных результатов необходимо строгое соблюдение Инструкции по применению Набора.

8.8. Не используйте компоненты из других наборов или из аналогичных наборов других серий.

9. ПРОВЕДЕНИЕ АНАЛИЗА

1	Поместите в рамку необходимое количество стрипов – исследуемые образцы в 2 повторах и 12 лунок для калибровочных проб и контрольной сыворотки.
2	**Разбавьте образцы сыворотки (плазмы) крови в 5000 раз**, используя Буфер для разведения образцов **(SP271Z)**. Пример: в пробирку Разведение 1 (1:100) добавьте: 10 мкл образца + 990 мкл Буфера для разведения образцов. В другую пробирку Разведение 2 (1:5000) добавьте: 10 мкл Разведения 1 + 490 мкл Буфера для разведения образцов. Разведение 2 (1:5000) следует использовать в анализе. Способ разведения для других видов материала приведен в таблице М. Не разбавляйте калибровочные пробы и контрольную сыворотку.
3	Если предполагаемая концентрация общего IgG в исследуемом образце превышает 25 г/л, его следует дополнительно развести, используя Буфер для разведения образцов (SP271Z). Использование других буферов и реагентов для разбавления образцов может искажать результаты определения! Примечание. Для получения надежных результатов рекомендуется использовать несколько последовательных разведений исследуемого образца биологических жидкостей.
4	**Внесите в соответствующие лунки в дубликатах по 100 мкл каждой калибровочной пробы и контрольной сыворотки**. При исследовании сыворотки (плазмы) крови в лунки, предназначенные для исследуемых образцов, **внесите по 100 мкл разбавленных образцов (Разведение 2)**. При исследовании других видов материала объем вносимого исследуемого образца указан в таблице М. Внесение калибровочных проб, контрольной сыворотки и исследуемых образцов необходимо произвести в течение 15 минут.
5	Аккуратно перемешайте содержимое планшета круговыми движениями по горизонтальной поверхности, заклейте планшет бумагой для заклеивания планшета. **Инкубируйте планшет в течение 30 минут при температуре +37 °С**.
6	По окончании инкубации удалите содержимое лунок аспирацией (например, с помощью водоструйного насоса) или декантированием и **отмойте лунки 3 раза**. При каждой отмывке добавьте во все лунки по 250 мкл отмывочного раствора (см. п. 7.3), встряхните планшет круговыми движениями по горизонтальной поверхности с последующей аспирацией или декантированием. Задержка при отмывке (замачивание лунок) не требуется. При каждом декантировании необходимо тщательно удалять остатки жидкости из лунок.
7	**Внесите во все лунки по 100 мкл конъюгата**.
8	Заклейте планшет бумагой для заклеивания планшета и **инкубируйте** его **в течение 30 минут при температуре +37 °С**.
9	По окончании инкубации удалите содержимое лунок и **отмойте лунки 5 раз**.
10	**Внесите во все лунки по 100 мкл раствора субстрата тетраметилбензидина**. Внесение раствора субстрата тетраметилбензидина в лунки необходимо произвести в течение 2–3 мин. Инкубируйте планшет в темноте при комнатной температуре (+18...+25 °С) в течение 10–20 минут в зависимости от степени развития синего окрашивания.
11	**Внесите во все лунки** с той же скоростью и в той же последовательности, как и раствор субстрата тетраметилбензидина, **по 100 мкл стоп-реагента**, при этом содержимое лунок окрашивается в ярко-желтый цвет.
12	**Измерьте величину оптической плотности (ОП) содержимого лунок планшета на фотометре** вертикального сканирования **при длине волны 450 нм**. Измерение ОП содержимого лунок планшета необходимо произвести в течение 15 мин после внесения стоп-реагента. Бланк фотометра выставляйте по калибровочной пробе С1.

продолжение таблицы на стр. 8

13	Постройте в линейных координатах калибровочный график: ось абсцисс (x) – концентрация общего IgG в калибровочных пробах (г/л), ось ординат (y) – оптическая плотность калибровочных проб (ОП 450 нм). Для алгоритма обсчета (аппроксимации) калибровочного графика используйте интервальный (кусочно-линейный, «от точки к точке») метод.
14	Определите по калибровочному графику содержание общего IgG в исследуемых образцах. Если исследуемый образец предразводили (см. п. 3), умножьте полученный результат на фактор разведения. При анализе различных видов материала необходимо умножить полученные значения на Фактор пересчета, приведенный в таблице М.

Таблица М

Вид материала	Сбор, хранение и обработка материала	Пример разведения	Буфер для разведения образцов лунку, мкл	Образец в лунку, мкл	Фактор пересчета
сыворотка (плазма) крови	Исследуемые образцы должны быть тщательно отцентрифугированы. Анализ мутных, хилезных и гемолитических образцов может привести к искажению результатов.	Разведение 1 (1:100): 10 мкл образца + 990 мкл Буфера для разведения образцов. В другую пробирку Разведение 2 (1:5000) добавьте 10 мкл Разведения 1 + 490 мкл Буфера для разведения образцов. Разведение 2 (1:5000) следует использовать в анализе	0	100	1
слюна	Исследуемые образцы должны быть тщательно отцентрифугированы. Анализ мутных образцов может привести к искажению результатов.		90	10	0.002
моча	Исследуемые образцы должны быть тщательно отцентрифугированы. Анализ мутных образцов может привести к искажению результатов.		50	50	0.0004
спинно-мозговая жидкость	Исследуемые образцы должны быть тщательно отцентрифугированы. Анализ мутных образцов может привести к искажению результатов.	10 мкл образца + 500 мкл буфера для разведения образцов	0	100	0.01

10. ОЖИДАЕМЫЕ ЗНАЧЕНИЯ И НОРМЫ

10.1. Основываясь на результатах исследований, проведенных ООО «ХЕМА», рекомендуем пользоваться нормами, приведенными ниже. Вместе с тем, в соответствии с правилами *GLP* (Хорошей лабораторной практики), каждая лаборатория должна сама определить параметры нормы, характерные для обследуемой популяции.

Примечание. Значения концентраций общего IgG в исследуемых образцах, находящиеся ниже границы чувствительности Набора (0.06 г/л), а также превышающие значение верхней калибровочной пробы (25 г/л) следует приводить в следующей форме: в исследуемом образце X концентрация общего IgG ниже 0.06 г/л или выше 25 г/л.

Исследуемая группа	Единицы, г/л	
	Нижний предел	Верхний предел
новорожденные	7.0	15
1-3 месяца	2.7	8.0
4-6 месяцев	1.8	8.5
7-12 месяцев	3.5	12
1-6 лет	6.5	18
7-11 лет	8.5	15
> 11 лет	9.0	20

11. ЛИТЕРАТУРА

1. RG Hamilton – Human IgG subclass measurements in the clinical laboratory. Clin. Chem., Oct 1987; 33: 1707 – 1725.
2. V. A. Semenova, E. Steward-Clark, K. L. Stamey, T. H. Taylor, Jr., D. S. Schmidt, S. K. Martin, N. Marano, and C. P. Quinn – Mass Value Assignment of Total and Subclass Immunoglobulin G in a Human Standard Anthrax Reference Serum. Clin. Diagn. Lab. Immunol., Sep 2004; 11: 919 – 923.

По вопросам, касающимся качества Набора **«общий IgG-ИФА»**,
следует обращаться в ООО «ХЕМА» по адресу:
105043, г. Москва, а/я 58
105264, г. Москва, ул. 9-я Парковая, д. 48, 1-й под., 5 этаж,
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электронная почта: info@xema.ru; rqc@xema.ru
интернет: www.xema.ru; www.xema-medica.com

Руководитель службы клиентского сервиса ООО «ХЕМА»,
к. б. н. Д. С. Кострикин

Instruction for use

A SOLID-PHASE ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF TOTAL IgG IN HUMAN BIOLOGICAL FLUIDS

1. INTENDED USE

A solid-phase enzyme immunoassay for the quantitative determination of total IgG in biological fluids.

This kit is designed for measurement of total IgG in biological fluids. For possibility of use with other sample types, please, refer to Application Notes (on request). The kit contains reagents sufficient for 96 determinations and allows to analyze 42 unknown samples in duplicates.

2. SUMMARY AND EXPLANATION

Immunoglobulin G (IgG) is the main part of serum γ – globulin fraction. IgG is secreted during secondary immune response and plays a key role in humoral immunity. Decrease of serum IgG concentration below 5 g/l is a marker of severe life-threatening immunodeficiency. Determination of serum IgG concentration and IgG/IgA/IgM ratios can be used for monitoring of humoral immune status. Marked elevation of serum IgG may be observed in chronic inflammation, autoimmune diseases and myeloma.

3. PRINCIPLE OF THE TEST

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific murine monoclonal to human total IgG-antibodies. Antigen from the specimen is captured by the antibodies coated onto the microwell surface. Unbound material is removed by washing procedure. Second antibodies – murine monocnoclonal to human total IgG, labelled with peroxidase enzyme, are then added into the microwells. After subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen.

4. WARNINGS AND PRECAUTIONS

4.1. For professional use only.

4.2. This kit is intended for in vitro diagnostic use only.

4.3. INFECTION HAZARD: There is no available test methods that can absolutely assure that Hepatitis B and C viruses, HIV-1/2, or other infectious agents are not present in the reagents of this kit. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

4.4. Avoid contact with stop solution containing 5.0% H_2SO_4. It may cause skin irritation and burns.

4.5. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents may give false results.

4.6. Do not use the kit beyond the expiration date.

4.7. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microplate readers.

4.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

4.9. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.

4.10. Do not mix reagents from different lots.

4.11. Replace caps on reagents immediately. Do not swap caps.

4.12. Do not pipette reagents by mouth.

4.13. Specimens must not contain any AZIDE compounds – they inhibit activity of peroxidase.

4.14. Material Safety Data Sheet for this product is available upon request directly from XEMA Co., Ltd.

4.15. The Material Safety Data Sheet fit the requirements of EU Guideline 91/155 EC.

5. KIT COMPONENTS

5.1. Contents of the Kit

	Symbol	Description		Qty	Units	Colour code	Stability of opened/diluted components
1	SORB MTP	total IgG EIA strips, 8x12 wells	polystyrene microwells coated with murine monoclonal to human total IgG	1	pcs		until exp.date
2	CAL 1–5	Calibrator set, 1 ml each. The set contains 5 calibrators: 0; 1; 5; 10; 25 g/l	human total IgG diluted in tris buffered BSA solution, preservative – 0.01% Bronidox L, 0.01% 2-Methyl-4-isothiazolin-3-one-hydrochloride; also contains blue dye	5	pcs	blue (C1 – colourless)	2 months
3	CONTROL	Control serum (1 ml)	dilution of preselected human serum, with high content of total IgG with BSA solution; preservative – 0.01% Bronidox L, 0.01% 2-Methyl-4-isothiazolin-3-one-hydrochloride, colourless	1	pcs	colourless	2 months
4	CONJ HRP	Conjugate, 14 ml	aqueous solution of murine monocnoclonal to human total IgG coupled with horseradish peroxidase diluted on phosphate buffered solution with casein from bovine milk and detergent (Tween-20), contains 0.1% phenol as preservative and red dye	1	pcs	red	until exp.date
5	DIL SPE	EIA sample buffer 100 ml	phosphate buffered saline with casein from bovine milk and detergent (Tween-20), contains 0.1% phenol as preservative and red dye	1	pcs	blue	until exp.date
6	SUBS TMB	Substrate solution, 14 ml	ready-to-use single-component tetramethylbenzidine (TMB) solution.	1	pcs	colourless	until exp.date
7	BUF WASH 26X	Washing solution concentrate 26X, 22 ml	aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative	1	pcs	colourless	Concentrate – until exp.date Diluted washing solution – 45 days at 2-8 °C or 15 days at RT
8	STOP	Stop solution, 14 ml	5.0% vol/vol solution of sulphuric acid	1	pcs	colourless	until exp.date
9	N003	Plate sealing tape		2	pcs		N/A
10	K271I	Instruction total IgG EIA		1	pcs		N/A
11	K271Q	QC data sheet total IgG EIA		1	pcs		N/A

5.2. Equipment and material required but not provided

- Distilled or deionized water;
- Automatic or semiautomatic multichannel micropipettes, 100–250 µl, is useful but not essential;
- Calibrated micropipettes with variable volume, range volume 10–250 µl;
- Dry thermostat for 37 °C ±0.1 °C
- Calibrated microplate photometer with 450 nm wavelength and OD measuring range 0–3.0

5.3. Storage and stability of the Kit

Store the whole kit at +2...+8 °C upon receipt until the expiration date.

After opening the pouch keep unused microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture.

6. SPECIMEN COLLECTION AND STORAGE

This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided.

Specimens may be stored for up to 48 hours at +2...+8 °C before testing.

7. TEST PROCEDURE

7.1. Reagent Preparation

- All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18...+25 °C) before use.
- All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.
- It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.
- Prepare washing solution from the concentrate BUF WASH 26X by 26 dilutions in distilled water.

7.2. Procedural Note:

It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

7.3. Assay flowchart

See the example of calibration graphic in Quality Control data sheet.

7.4. Assay procedure

1	Put the desired number of microstrips into the frame; allocate 12 wells for the calibrators CAL 1–5 and control samples CONTROL and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.
2	Dilute samples using buffer DIL SPE (EIA sample buffer) 5000 fold. See table M for dilution modes and factors for different types of analyzed material. Do not dilute control sample and calibrators.
3	If suggested analyte concentration in the sample exceeds the highest calibrator, additionally dilute this sample accordingly, using DIL SPE (EIA sample buffer). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.
4	Pipet 100 µl of calibrators CAL 1-5 and control samples CONTROL into allocated wells. For testing of blood serum or plasma pipet 100 µl of the unknown diluted sample (DILUTION 2) into the allocated wells. See table M for the volumes of other materials. Pipetting should be made within 3 minutes, to ensure an uniform incubation time for all samples. Carefully mix the contents of the wells by short horizontal rotating of the plate for 5-7 seconds and cover the wells by plate adhesive tape (included into the kit).
5	Incubate 30 minutes at +37 °C.
6	Prepare washing solution by 26X dilution of washing solution concentrate BUF WASH 26X by distilled water. Minimal quantity of washing solution should be 250 µl per well. Wash strips 3 times.
7	Dispense 100 µl of CONJ HRP into the wells. Cover the wells by plate adhesive tape.
8	Incubate 30 minutes at +37 °C .
9	Wash the strips 5 times.
10	Dispense 100 µl of SUBS TMB into the wells.
11	Incubate 10-20 minutes at +18...+25 °C.
12	Dispense 100 µl of STOP into the wells.
13	Measure OD (optical density) at 450 nm.
14	Set photometer blank on first calibrator.
15	Apply point-by-point method for data reduction. Use Calculation factor listed in table M to calculate analyte concentration in different material types.

7.5. Sample processing

Material type	Notes on material collection, storage and handling	Sample dilution example	EIA sample buffer into the well, µl	Sample into the well, µl	Calculation factor
blood serum or plasma	Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.	10 µl of sample + 990 µl of diluent = DILUTION 1. 10 µl of DILUTION1 + 490 µl of diluent = DILUTION 2	0	100	1
saliva	Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.		90	10	0.002
urine	Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.		50	50	0.0004
cerebrospinal fluid	Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.	10 µl of sample + 500 µl of diluent	0	100	0.01

8. QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state, and local standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications.

9. CALCULATION OF RESULTS

9.1. Calculate the mean absorbance values (OD450) for each pair of calibrators and samples.

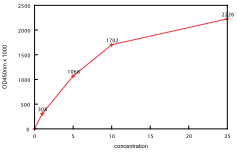
9.2. Plot a calibration curve on graph paper: OD versus total IgG concentration.

9.3. Determine the corresponding concentration of total IgG in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is

recommended due to non-linear shape of curve.

9.4. Below is presented a typical example of a standard curve with the XEMA Co. Not for calculations!

Calibrators	Value	Absorbance Units (450 nm)
CAL 1	0 g/l	0.08
CAL 2	1 g/l	0.38
CAL 3	5 g/l	1.15
CAL 4	10 g/l	1.78
CAL 5	25 g/l	2.30



10. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for total IgG. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

Sex, age	Units, g/l	
	Lower limit	Upper limit
newborn	7.0	15
1-3 month	2.7	8.0
4-6 month	1.8	8.5
7-12 month	3.5	12
1-6 yrs	6.5	18
7-11 yrs	8.5	15
> 11 yrs	9.0	20

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical specificity / Cross reactivity

Analyte	Cross-reactivity, % wt/wt
IgA	<0.1
IgM	<0.1
IgE	<0.1

11.2. Analytical sensitivity. Sensitivity of the assay was assessed as being 0.06 g/l.

11.3. Linearity. Linearity was checked by assaying dilution series of 5 samples with different total IgG concentrations. Linearity percentages obtained ranged within 90 to 110%.

11.4. Recovery. Recovery was estimated by assaying 5 mixed samples with known total IgG concentrations. The recovery percentages ranged from 90 to 110%.

12. LITERATURE

1. RG Hamilton – Human IgG subclass measurements in the clinical laboratory. Clin. Chem., Oct 1987; 33: 1707 – 1725.

2. V. A. Semenova, E. Steward-Clark, K. L. Stamey, T. H. Taylor, Jr., D. S. Schmidt, S. K. Martin, N. Marano, and C. P. Quinn – Mass Value Assignment of Total and Subclass Immunoglobulin G in a Human Standard Anthrax Reference Serum. Clin. Diagn. Lab. Immunol., Sep 2004; 11: 919 – 923.

Символ / Symbol	Значение символа / Symbolize
	Производитель / Manufacturer
	Дата производства / Date of manufacture
REF	Номер по каталогу / Catalogue number
LOT	Номер серии / Batch code
 YYYY-MM	Использовать до (год-месяц) / Use By
	Ограничение температуры / Temperature limitation
IVD	Только для ин витро диагностики / In Vitro Diagnostic Medical Device
	Внимание! / Caution, consult accompanying documents
	Не использовать при нарушении целостности упаковки / Do not use if package damaged
SORB MTP	Планшет / EIA strips
CAL	Калибровочные пробы / Calibrator set
CONTROL	Контрольная сыворотка / Control sera
CONJ HRP	Конъюгат / Conjugate
SUBS TMB	Раствор субстрата тетраметилбензидина (ТМБ) / Substrate solution
BUF WASH 26X	Концентрат отмывочного раствора / Washing solution concentrate
STOP	Стоп-реагент / Stop solution
DIL	ИФА-Буфер / EIA buffer

Уважаемый Клиент!
Если в процессе работы с нашими Наборами Вам понадобились пластиковые ванночки для жидких реагентов, одноразовые наконечники для дозаторов или дополнительные объемы реагентов (концентрат отмывочного раствора, ИФА-Буфер, раствор субстрата тетраметилбензидина (ТМБ), стоп-реагент), входящих в состав Набора, просим Вас обратиться к поставщику продукции ООО «ХЕМА» в Вашем регионе.
Все указанные расходные материалы предоставляются бесплатно, в необходимом для проведения анализа количестве.

Перечень Наборов реагентов для диагностики инфекционных заболеваний производства ООО «ХЕМА»

№ по каталогу	Наименование
K101	«Toxoplasma IgG-ИФА»
K101M	«Toxoplasma IgM-ИФА»
K102	«Rubella IgG-ИФА»
K102M	«Rubella IgM-ИФА»
K103	«Cytomegalovirus IgG-ИФА»
K103M	«Cytomegalovirus IgM-ИФА»
K104	«HSV 1,2 IgG-ИФА»
K104M	«HSV 1,2 IgM-ИФА»
K105	«Chlamydia IgG-ИФА»
K106	«Mycoplasma IgG-ИФА»
K111G	«Сифилис IgG-ИФА»
K111	«Сифилис суммарные антитела-ИФА»
K121	«Aspergillus IgG-ИФА»

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xemahelp

xemahelp@gmail.com

Instruction for use

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ НАБОРА РЕАГЕНТОВ ДЛЯ ИММУНОФЕРМЕНТНОГО ОПРЕДЕЛЕНИЯ ОБЩЕГО IgA В БИОЛОГИЧЕСКИХ ЖИДКОСТЯХ

«Общий IgA-ИФА»

A SOLID-PHASE ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF TOTAL IgA IN HUMAN BIOLOGICAL FLUIDS

Total IgA EIA

НОМЕР ПО КАТАЛОГУ REF **K275**

ТУ № 9398-275-18619450-2009

РЕГИСТРАЦИОННОЕ УДОСТОВЕРЕНИЕ
№ ФСР 2009/06103 от 19 ноября 2009 г.

For 96 determinations/На 96 определений

IVD Для *ин витро* диагностики

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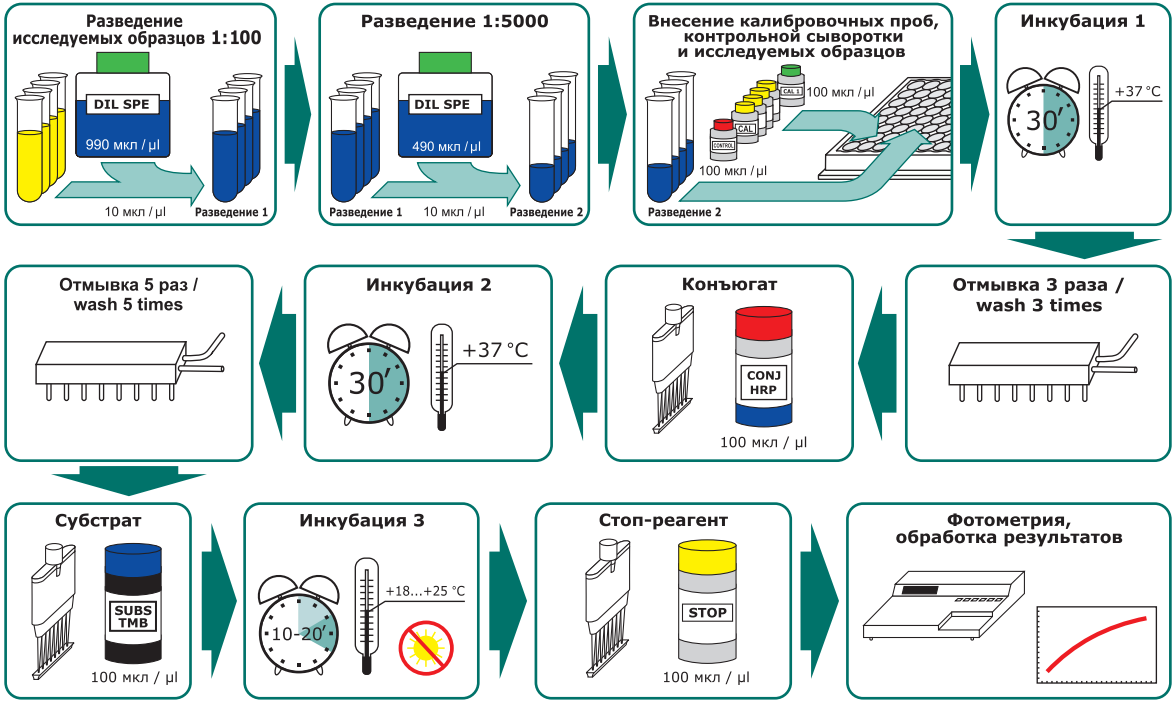
Telephone/fax: +7(495) 737-39-36; 737-00-40
e-mail: redkin@xema-medica.com
internet: www.xema-medica.com

EC REP

Authorized Representative in EU:
Polmed.de
Steinacker 20, D-73773
Aichwald, Germany
e-mail: info@polmed.de

Схема проведения анализа / Test procedure *



K271, K274, K275, K277

* Для сыворотки (плазмы) крови.
Способ разведения для других видов материала приведен в таблице М

* Blood serum or plasma
For other tested materials, see table M.

СОДЕРЖАНИЕ

CONTENT

Инструкция составлена Руководителем службы клиентского сервиса ООО «ХЕМА», к. б. н. Д. С. Кострикиным

«УТВЕРЖДЕНА»
Приказ Росздравнадзора № 9363-Пр/09 от 19 ноября 2009 г.
КРД 68431 от 24.09.2009 г.

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ НАБОРА РЕАГЕНТОВ ДЛЯ ИММУНОФЕРМЕНТНОГО ОПРЕДЕЛЕНИЯ ОБЩЕГО IgA В БИОЛОГИЧЕСКИХ ЖИДКОСТЯХ «общий IgA-ИФА»

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов «общий IgA-ИФА» предназначен для количественного определения концентрации общего IgA в биологических жидкостях (см. таблицу М) методом твердофазного иммуноферментного анализа.

1.2. Иммуноглобулин А (IgA) основной гуморальный фактор иммунной защиты слизистых оболочек. Один из наиболее часто встречающихся в популяции врожденных дефектов – это селективный IgA дефицит. Селективный дефицит IgA приводит к синдрому хронических инфекционных заболеваний желудочно-кишечного тракта, мочевыводящих и дыхательных путей. Определение концентрации IgA в сыворотке крови, а также в других биологических жидкостях может использоваться в качестве основного скринингового теста для оценки гуморального иммунного статуса индивида. Резкое повышение сывороточной концентрации IgA характерно для некоторых аутоиммунных заболеваний и миеломной болезни.

2. ПРИНЦИП РАБОТЫ НАБОРА

Определение общего IgA основано на использовании «сэндвич»-варианта твердофазного иммуноферментного анализа. На внутренней поверхности лунок планшета иммобилизованы мышиные моноклональные антитела к общему IgA человека. В лунках планшета, при добавлении исследуемого образца, происходит связывание общего IgA, содержащегося в исследуемом образце, с антителами на твердой фазе. Образовавшийся комплекс выявляют с помощью конъюгата мышиных моноклональных антител к общему IgA с пероксидазой хрена. В результате образуется связанный с пластиком «сэндвич», содержащий пероксидазу. Во время инкубации с раствором субстрата тетраметилбензидина (ТМБ) происходит окрашивание растворов в лунках. Интенсивность окраски прямо пропорциональна концентрации общего IgA в исследуемом образце. Концентрацию общего IgA в исследуемых образцах определяют по калибровочному графику зависимости оптической плотности от содержания общего IgA в калибровочных пробах.

3. АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Специфичность. Перекрестная реакция мышиных моноклональных антител к общему IgA с другими аналитами приведена в таблице:

Аналит	Перекрестная реакция, %
IgG	<0.1
IgM	<0.1
IgE	<0.1

3.2. Воспроизводимость.

Коэффициент вариации результатов определения содержания общего IgA в одном и том же образце биологических жидкостей с использованием Набора «общий IgA-ИФА» не превышает 8.0%.

3.3. Линейность.

Зависимость концентрации общего IgA в образцах биологических жидкостей при разведении их биологическими жидкостями, не содержащей общий IgA, имеет линейный характер в диапазоне концентраций 0.1–5 г/л и составляет ±10.0%.

3.4. Точность.

Данный аналитический параметр проверяется тестом на «открытие» – соответствие измеренной концентрации общего IgA предписанной, полученной путем смешивания равных объемов контрольной сыворотки и калибровочной пробы 0.5 г/л. Процент «открытия» составляет 90–110%.

3.5. Чувствительность.

Минимальная достоверно определяемая Набором «общий IgA-ИФА» концентрация общего IgA в биологических жидкостях не превышает 0.06 г/л.

4. СОСТАВ НАБОРА

	Код компонента	Символ	Наименование	Кол-во	Ед.	Описание
1	P275Z	SORB MTP	**Планшет** 96-луночный полистироловый, стрипированный, готов к использованию	1	шт.	-
2	C275Z	CAL 1–5	**Калибровочные пробы** на основе трис-буфера (pH 7.2–7.4), содержащие известные количества общего IgA – **0**; **0.1**; **0.5**; **2**; **5 г/л**, готовы к использованию (по 1 мл каждая)	5	шт.	прозрачные жидкости синего цвета (калибровочная проба 0 – прозрачная бесцветная жидкость)
3	Q275Z	CONTROL	**Контрольная сыворотка** на основе сыворотки крови человека с известным содержанием общего IgA, готова к использованию (1 мл)	1	шт.	прозрачная бесцветная жидкость
4	T275Z	CONJ HRP	**Конъюгат**, готов к использованию (14 мл)	1	шт.	прозрачная жидкость синего цвета
5	S011Z4	DIL SPE	**ИФА-Буфер**, готов к использованию (100 мл)	1	шт.	прозрачная жидкость синего цвета
6	R055Z	SUBS TMB	**Раствор субстрата тетраметилбензидина** (ТМБ), готов к использованию (14 мл)	1	шт.	прозрачная бесцветная жидкость
7	S008Z	BUF WASH 26X	**Концентрат отмывочного раствора** (солевой раствор с твин-20 и бензойной кислотой), 26х-кратный (22 мл)	1	шт.	прозрачная бесцветная жидкость
8	R050Z	STOP	**Стоп-реагент**, готов к использованию (14 мл)	1	шт.	прозрачная бесцветная жидкость
9	N003	-	Бумага для заклеивания планшета	2	шт.	-
10	K275I	-	Инструкция по применению Набора реагентов «общий IgA-ИФА»	1	шт.	-
11	K275Q	-	Паспорт контроля качества Набора реагентов «общий IgA-ИФА»	1	шт.	-

5. МЕРЫ ПРЕДОСТОРОЖНОСТИ

5.1. Потенциальный риск применения Набора – класс 2а (ГОСТ Р 51609-2000).

5.2. Все компоненты Набора, за исключением стоп-реагента (5.0% раствор серной кислоты), в используемых концентрациях являются нетоксичными.

Раствор серной кислоты обладает раздражающим действием. Избегать разбрызгивания и попадания на кожу и слизистые. При попадании на кожу и слизистые пораженный участок следует промыть большим количеством проточной воды.

5.3. При работе с Набором следует соблюдать «Правила устройства, техники безопасности, производственной санитарии, противоэпидемического режима и личной гигиены при работе в лабораториях (отделениях, отделах) санитарно-эпидемиологических учреждений системы Министерства здравоохранения СССР» (Москва, 1981 г.).

5.4. При работе с Набором следует надевать одноразовые резиновые или пластиковые перчатки, так как образцы крови человека следует рассматривать как потенциально инфицированный материал, способный длительное время сохранять и передавать ВИЧ, вирус гепатита или любой другой возбудитель вирусной инфекции.

6. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ПРИ РАБОТЕ С НАБОРОМ

- фотометр вертикального сканирования, позволяющий измерять оптическую плотность содержимого лунок планшета при длине волны 450 нм;
- термостат, поддерживающий температуру +37 °С ±0.1 °С;
- дозаторы со сменными наконечниками, позволяющие отбирать объемы в диапазоне 5–250 мкл;
- цилиндр мерный вместимостью 1000 мл;
- вода дистиллированная;
- перчатки резиновые или пластиковые;
- бумага фильтровальная.

7. ПОДГОТОВКА РЕАГЕНТОВ ДЛЯ АНАЛИЗА

7.1. Перед проведением анализа компоненты Набора и исследуемые образцы сыворотки (плазмы) крови следует выдержать при комнатной температуре (+18...+25 °С) не менее 30 мин.

7.2. Приготовление планшета.

Вскрыть пакет с планшетом и установить на рамку необходимое количество стрипов. Оставшиеся неиспользованными стрипы, чтобы предотвратить воздействие на них влаги, тщательно заклеить бумагой для заклеивания планшета и хранить при температуре +2...+8 °С в течение всего срока годности Набора.

7.3. Приготовление отмывочного раствора.

Содержимое флакона с концентратом отмывочного раствора (22 мл), перенести в мерный цилиндр вместимостью 1000 мл, добавить 550 мл дистиллированной воды и тщательно перемешать. В случае дробного использования Набора следует отобрать необходимое количество концентрата отмывочного раствора и развести дистиллированной водой в 26 раз (1 мл концентрата отмывочного раствора + 25 мл дистиллированной воды).

8. УСЛОВИЯ ХРАНЕНИЯ И ЭКСПЛУАТАЦИИ НАБОРА

8.1. Набор реагентов «общий IgA-ИФА» должен храниться в упаковке предприятия-изготовителя при температуре +2...+8 °С в течение всего срока годности, указанного на упаковке Набора.

Допускается хранение (транспортировка) Набора при температуре до +25 °С не более 15 суток. Не допускается замораживание целого набора.

8.2. Набор рассчитан на проведение анализа в дубликатах 42 исследуемых образцов, 5 калибровочных проб и 1 пробы контрольной сыворотки (всего 96 определений).

8.3. В случае дробного использования Набора компоненты следует хранить следующим образом:

- оставшиеся неиспользованными стрипы необходимо тщательно заклеить бумагой для заклеивания планшета и хранить при температуре +2...+8 °С в течение всего срока годности Набора;
- ИФА-Буфер, конъюгат, субстрат, стоп-реагент после вскрытия флаконов следует хранить при температуре +2...+8 °С в течение всего срока годности Набора;
- калибровочные пробы и контрольную сыворотку после вскрытия флаконов следует хранить при температуре +2...+8 °С не более 2 месяцев;
- оставшийся неиспользованным концентрат отмывочного раствора следует хранить при температуре +2…+8 °С в течение всего срока годности Набора. Приготовленный отмывочный раствор следует хранить при комнатной температуре (+18…+25 °С) не более 15 суток или при температуре +2…+8 °С не более 45 суток.

Примечание. После использования реагента немедленно закрывайте крышку флакона. Закрывайте каждый флакон своей крышкой.

8.4. Для проведения анализа не следует использовать гемолизированную, мутную сыворотку (плазму) крови, а также сыворотку (плазму) крови, содержащую азид натрия. Если анализ производится не в день взятия крови, сыворотку (плазму) следует хранить при температуре -20 °С. Повторное замораживание-оттаивание образцов сыворотки (плазмы) крови не допускается.

8.5. Исключается использование для анализа образцов сыворотки (плазмы) крови людей, получавших в целях диагностики или терапии препараты, в состав которых входят мышиные антитела.

8.6. При использовании Набора для проведения нескольких независимых серий анализов следует иметь в виду, что для каждого независимого определения необходимо построение нового калибровочного графика; кроме этого, рекомендуется определение концентрации общего IgA в контрольной сыворотке.

8.7. Для получения надежных результатов необходимо строгое соблюдение Инструкции по применению Набора.

8.8. Не используйте компоненты из других наборов или из аналогичных наборов других серий.

9. ПРОВЕДЕНИЕ АНАЛИЗА

1	Поместите в рамку необходимое количество стрипов – исследуемые образцы в 2 повторах и 12 лунок для калибровочных проб и контрольной сыворотки.
2	Разбавьте образцы сыворотки (плазмы) крови в 5000 раз, используя ИФА-Буфер. Пример: в пробирку Разведение 1 (1:100): 10 мкл образца + 990 мкл ИФА-Буфера. В другую пробирку Разведение 2 (1:5000) добавьте 10 мкл Разведения 1 + 490 мкл ИФА-Буфера. Разведение 2 (1:5000) следует использовать в анализе. Способ разведения для других видов материала приведен в таблице М. Не разбавляйте калибровочные пробы и контрольную сыворотку.
3	Если предполагаемая концентрация общего IgA в исследуемом образце превышает 5 г/л, его следует дополнительно развести, используя ИФА-Буфер. Использование других буферов и реагентов для разбавления образцов может искажать результаты определения! Примечание. Для получения надежных результатов рекомендуется использовать несколько последовательных разведений исследуемого образца биологических жидкостей.
4	**Внесите в соответствующие лунки в дубликатах по 100 мкл каждой калибровочной пробы и контрольной сыворотки**. При исследовании сыворотки (плазмы) крови в лунки, предназначенные для исследуемых образцов, **внесите по 100 мкл разбавленных образцов (Разведение 2)**. При исследовании других видов материала объем вносимого исследуемого образца указан в таблице М. Внесение калибровочных проб, контрольной сыворотки и исследуемых образцов необходимо произвести в течение 15 минут.
5	Аккуратно перемешайте содержимое планшета круговыми движениями по горизонтальной поверхности, заклейте планшет бумагой для заклеивания планшета. **Инкубируйте планшет в течение 30 минут при температуре +37 °С**.
6	По окончании инкубации удалите содержимое лунок аспирацией (например, с помощью водоструйного насоса) или декантированием и **отмойте лунки 3 раза**. При каждой отмывке добавьте во все лунки по 250 мкл отмывочного раствора (см. п. 7.3), встряхните планшет круговыми движениями по горизонтальной поверхности с последующей аспирацией или декантированием. Задержка при отмывке (замачивание лунок) не требуется. При каждом декантировании необходимо тщательно удалять остатки жидкости из лунок.
7	**Внесите во все лунки по 100 мкл конъюгата**.
8	Заклейте планшет бумагой для заклеивания планшета и **инкубируйте** его **в течение 30 минут при температуре +37 °С**.
9	По окончании инкубации удалите содержимое лунок и **отмойте лунки 5 раз**.
10	**Внесите во все лунки по 100 мкл раствора субстрата тетраметилбензидина**. Внесение раствора субстрата тетраметилбензидина в лунки необходимо произвести в течение 2–3 мин. **Инкубируйте планшет в темноте при комнатной температуре (+18...+25 °С) в течение 10–20 минут** в зависимости от степени развития синего окрашивания.
11	**Внесите во все лунки** с той же скоростью и в той же последовательности, как и раствор субстрата тетраметилбензидина, **по 100 мкл стоп-реагента**, при этом содержимое лунок окрашивается в ярко-желтый цвет.
12	**Измерьте величину оптической плотности (ОП) содержимого лунок планшета на фотометре** вертикального сканирования **при длине волны 450 нм**. Измерение ОП содержимого лунок планшета необходимо произвести в течение 15 мин после внесения стоп-реагента. Бланк фотометра выставляйте по калибровочной пробе С1.

продолжение таблицы на стр. 8

13	Постройте в линейных координатах калибровочный график: ось абсцисс (x) – концентрация общего IgA в калибровочных пробах (г/л), ось ординат (y) – оптическая плотность калибровочных проб (ОП 450 нм). Для алгоритма обсчета (аппроксимации) калибровочного графика используйте интервальный (кусочно-линейный, «от точки к точке») метод.
14	Определите по калибровочному графику содержание общего IgA в исследуемых образцах. Если исследуемый образец предразводили (см. п.3), умножьте полученный результат на фактор разведения. При анализе различных видов материала необходимо умножить полученные значения на Фактор пересчета, приведенный в таблице М.

Таблица М

Вид материала	Сбор, хранение и обработка материала	Пример разведения	ИФА-Буфер в лунку, мкл	Образец в лунку, мкл	Фактор пере-счета
сыворотка (плазма) крови	Исследуемые образцы должны быть тщательно отцентрифугированы. Анализ мутных, хилезных и гемолитических образцов может привести к искажению результатов.	Разведение 1 (1:100): 10 мкл образца + 990 мкл ИФА-Буфера. В другую пробирку Разведение 2 (1:5000) добавьте 10 мкл Разведения 1 + 490 мкл ИФА-Буфера. Разведение 2 (1:5000) следует использовать в анализе	0	100	1
слюна	Исследуемые образцы должны быть тщательно отцентрифугированы. Анализ мутных образцов может привести к искажению результатов.	5 мкл образца + 500 мкл ИФА-Буфера	90	10	0.2
моча	Исследуемые образцы должны быть тщательно отцентрифугированы. Анализ мутных образцов может привести к искажению результатов.		80	20	0.001
спинно-мозговая жидкость	Исследуемые образцы должны быть тщательно отцентрифугированы. Анализ мутных образцов может привести к искажению результатов.		50	50	0.0004

10. ОЖИДАЕМЫЕ ЗНАЧЕНИЯ И НОРМЫ

10.1. Основываясь на результатах исследований, проведенных ООО «ХЕМА», рекомендуем пользоваться нормами, приведенными ниже. Вместе с тем, в соответствии с правилами *GLP* (Хорошей лабораторной практики), каждая лаборатория должна сама определить параметры нормы, характерные для обследуемой популяции.

Примечание. Значения концентраций общего IgA в исследуемых образцах, находящиеся ниже границы чувствительности Набора (0.06 г/л), а также превышающие значение верхней калибровочной пробы (5 г/л) следует приводить в следующей форме: в исследуемом образце X концентрация общего IgA ниже 0.06 г/л или выше 5 г/л.

Исследуемая группа	Единицы, г/л	
	Нижний предел	Верхний предел
Здоровые доноры	0.9	5.0
>61 года	1.0	6.5
новорожденные	-	0.05
1-3 месяца	0.06	0.6
4-6 месяцев	0.1	1.0
7-12 месяцев	0.35	1.7
1-6 лет	0.8	2.2
7-11 лет	0.9	2.6

11. ЛИТЕРАТУРА

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2. Amir H Abdul Latiff and Michael A Kerr – The clinical significance of immunoglobulin A deficiency. Ann Clin Biochem, Mar 2007; 44: 131 – 139.

По вопросам, касающимся качества Набора **«общий IgA-ИФА»**,
следует обращаться в ООО «ХЕМА» по адресу:
105043, г. Москва, а/я 58
105264, г. Москва, ул. 9-я Парковая, д. 48, 1-й под., 5 этаж,
тел/факс (495) 737-39-36, 737-00-40, 510-57-07 (многоканальный)

электронная почта: info@xema.ru; rqc@xema.ru
интернет: www.xema.ru; www.xema-medica.com

Руководитель службы клиентского сервиса ООО «ХЕМА»,
к. б. н. Д. С. Кострикин

Instruction for use

A SOLID-PHASE ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF TOTAL IGA IN HUMAN BIOLOGICAL FLUIDS

1. INTENDED USE

A solid-phase enzyme immunoassay for the quantitative determination of total IgA in biological fluids.

This kit is designed for measurement of total IgA in biological fluids. For possibility of use with other sample types, please, refer to Application Notes (on request). The kit contains reagents sufficient for 96 determinations and allows to analyze 42 unknown samples in duplicates.

2. SUMMARY AND EXPLANATION

Immunoglobulin A (IgA) is a main factor of mucosal immune response to bacteria and viruses. Selective IgA deficiency is one of the most frequent hereditary disorders causing chronic infections inflammation in gastrointestinal, urinary and respiratory systems. Determination of IgA concentration in serum and other biological fluids can be used as screening for selective IgA deficiency and other immunodeficiency syndromes. Marked elevation of serum IgA is observed in some autoimmune diseases and IgA myeloma.

3. PRINCIPLE OF THE TEST

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific murine monoclonal to human total IgA-antibodies. Antigen from the specimen is captured by the antibodies coated onto the microwell surface. Unbound material is removed by washing procedure. Second antibodies – murine monoclonal to human total IgA, labelled with peroxidase enzyme, are then added into the microwells. After subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen.

4. WARNINGS AND PRECAUTIONS

4.1. For professional use only.

4.2. This kit is intended for in vitro diagnostic use only.

4.3. INFECTION HAZARD: There is no available test methods that can absolutely assure that Hepatitis B and C viruses, HIV-1/2, or other infectious agents are not present in the reagents of this kit. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

4.4. Avoid contact with stop solution containing 5.0% H_2SO_4. It may cause skin irritation and burns.

4.5. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents may give false results.

4.6. Do not use the kit beyond the expiration date.

4.7. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microplate readers.

4.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

4.9. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.

4.10. Do not mix reagents from different lots.

4.11. Replace caps on reagents immediately. Do not swap caps.

4.12. Do not pipette reagents by mouth.

4.13. Specimens must not contain any AZIDE compounds – they inhibit activity of peroxidase.

4.14. Material Safety Data Sheet for this product is available upon request directly from XEMA Co., Ltd.

4.15. The Material Safety Data Sheet fit the requirements of EU Guideline 91/155 EC.

5. KIT COMPONENTS

5.1. Contents of the Kit

	Symbol	Description		Qty	Units	Colour code	Stability of opened/diluted components
1	SORB MTP	total IgA EIA strips, 8x12 wells	polystyrene microwells coated with murine monoclonal to human total IgA	1	pcs		until exp.date
2	CAL 1–5	Calibrator set, 1 ml each. The set contains 5 calibrators: 0; 0.1; 0.5; 2; 5 g/l	human total IgA diluted in tris buffered BSA solution, preservative – 0.01% Bronidox L, 0.01% 2-Methyl-4-isothiazolin-3-one-hydrochloride; also contains blue dye	5	pcs	blue (C1 – colourless)	2 months
3	CONTROL	Control serum (1 ml)	dilution of preselected human serum, with high content of total IgA with BSA solution; preservative - 0.01% Bronidox L, 0.01% 2-Methyl-4-isothiazolin-3-one-hydrochloride, colourless	1	pcs	colourless	2 months
4	CONJ HRP	Conjugate, 14 ml	aqueous solution of murine monoclonal to human total IgA coupled with horseradish peroxidase diluted on phosphate buffered solution with casein from bovine milk and detergent (Tween-20), contains 0.1% phenol as preservative and blue dye	1	pcs	blue	until exp.date
5	DIL SPE	EIA buffer 100 ml	phosphate buffered saline with casein from bovine milk and detergent (Tween-20), contains 0.1% phenol as preservative and blue dye	1	pcs	blue	until exp.date
6	SUBS TMB	Substrate solution, 14 ml	ready-to-use single-component tetramethylbenzidine (TMB) solution.	1	pcs	colourless	until exp.date
7	BUF WASH 26X	Washing solution concentrate 26X, 22 ml	aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative	1	pcs	colourless	Concentrate – until exp.date Diluted washing solution - 45 days at 2-8 °C or 15 days at RT
8	STOP	Stop solution, 14 ml	5.0% vol/vol solution of sulphuric acid	1	pcs	colourless	until exp.date
9	N003	Plate sealing tape		2	pcs		N/A
10	K275I	Instruction total IgA EIA		1	pcs		N/A
11	K275Q	QC data sheet total IgA EIA		1	pcs		N/A

5.2. Equipment and material required but not provided

- Distilled or deionized water;
- Automatic or semiautomatic multichannel micropipettes, 100–250 µl, is useful but not essential;
- Calibrated micropipettes with variable volume, range volume 5–250 µl;
- Dry thermostat for +37 °C ±0.1 °C
- Calibrated microplate photometer with 450 nm wavelength and OD measuring range 0–3.0

5.3. Storage and stability of the Kit

Store the whole kit at +2...+8 °C upon receipt until the expiration date.

After opening the pouch keep unused microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture.

6. SPECIMEN COLLECTION AND STORAGE

This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided.

Specimens may be stored for up to 48 hours at +2...+8 °C before testing.

7. TEST PROCEDURE

7.1. Reagent Preparation

- All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18...+25 °C) before use.
- All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.
- It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.
- Prepare washing solution from the concentrate BUF WASH 26X by 26 dilutions in distilled water.

7.2. Procedural Note:

It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

7.3. Assay flowchart

See the example of calibration graphic in Quality Control data sheet.

7.4. Assay procedure

1	Put the desired number of microstrips into the frame; allocate 12 wells for the calibrators CAL 1–5 and control samples CONTROL and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.
2	Dilute samples using buffer DIL SPE (EIA buffer) 5000 fold. See table M for dilution modes and factors for different types of analyzed material. Do not dilute control sample and calibrators.
3	If suggested analyte concentration in the sample exceeds the highest calibrator, additionally dilute this sample accordingly, using DIL SPE (EIA buffer). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.
4	Pipet 100 µl of calibrators CAL 1–5 and control samples CONTROL into allocated wells. For testing of blood serum or plasma pipet 100 µl of the diluted unknown sample into the allocated wells. See table M for the volumes of other materials. Pipetting should be made within 3 minutes, to ensure an uniform incubation time for all samples. Carefully mix the contents of the wells by short horizontal rotating of the plate for 5-7 seconds and cover the wells by plate adhesive tape (included into the kit).
5	Incubate 30 minutes at +37 °C.
6	Prepare washing solution by 26X dilution of washing solution concentrate BUF WASH 26X by distilled water. Minimal quantity of washing solution should be 250 µl per well. Wash strips 3 times.
7	Dispense 100 µl of CONJ HRP into the wells.
8	Incubate 30 minutes at +37 °C.
9	Wash the strips 5 times.
10	Dispense 100 µl of SUBS TMB into the wells.
11	Incubate 10-20 minutes at +18...+25 °C.
12	Dispense 100 µl of STOP into the wells.
13	Measure OD (optical density) at 450 nm.
14	Set photometer blank on first calibrator.
15	Apply point-by-point method for data reduction. Use Calculation factor listed in table M to calculate analyte concentration in different material types.

7.5. Sample processing

Material type	Notes on material collection, storage and handling	Sample dilution example	EIA buffer into the well, µl	Sample into the well, µl	Calculation factor
blood serum or plasma	Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.	10 µl of sample + 990 µl of diluent = DILUTION 1. 10 µl of DILUTION1 + 490 µl of diluent = DILUTION 2	0	100	1
saliva	Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.	5 µl of sample + 500 µl of diluent	90	10	0.2
urine	Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.		80	20	0.001
cerebrospinal fluid	Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.		50	50	0.0004

8. QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state, and local standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications.

9. CALCULATION OF RESULTS

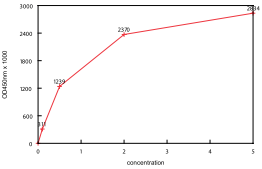
9.1. Calculate the mean absorbance values (OD450) for each pair of calibrators and samples.

9.2. Plot a calibration curve on graph paper: OD versus total IgA concentration.

9.3. Determine the corresponding concentration of total IgA in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

9.4. Below is presented a typical example of a standard curve with the XEMA Co. Not for calculations!

Calibrators	Value	Absorbance Units (450 nm)
CAL 1	0 g/l	0.08
CAL 2	0.1 g/l	0.40
CAL 3	0.5 g/l	1.32
CAL 4	2 g/l	2.45
CAL 5	5 g/l	2.92



10. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for total IgA. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

Sex, age	Units, g/l	
	Lower limit	Upper limit
Healthy donors	0.9	5.0
>61 yr	1.0	6.5
newborn	-	0.05
1-3 month	0.06	0.6
4-6 month	0.1	1.0
7-12 month	0.35	1.7
1-6 yrs	0.8	2.2
7-11 yrs	0.9	2.6

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical specificity / Cross reactivity

Analyte	Cross-reactivity, % wt/wt
IgG	<0.1
IgM	<0.1
IgE	<0.1

11.2. Analytical sensitivity. Sensitivity of the assay was assessed as being 0.06 g/l.

11.3. Linearity. Linearity was checked by assaying dilution series of 5 samples with different total IgA concentrations. Linearity percentages obtained ranged within 90 to 110%.

11.4. Recovery. Recovery was estimated by assaying 5 mixed samples with known total IgA concentrations. The recovery percentages ranged from 90 to 110%.

12. LITERATURE

1. Heiddis B. Valdimarsdottir and Arthur A. Stone – Psychosocial Factors and Secretory Immunoglobulin A. Critical Reviews in Oral Biology & Medicine, Jan 1997; 8: 461 – 474.

2. Amir H Abdul Latiff and Michael A Kerr – The clinical significance of immunoglobulin A deficiency. Ann Clin Biochem, Mar 2007; 44: 131 – 139.

Символ / Symbol	Значение символа / Symbolize
	Производитель / Manufacturer
	Дата производства / Date of manufacture
REF	Номер по каталогу / Catalogue number
LOT	Номер серии / Batch code
 YYYY-MM	Использовать до (год-месяц) / Use By
	Ограничение температуры / Temperature limitation
IVD	Только для ин витро диагностики / In Vitro Diagnostic Medical Device
	Внимание! / Caution, consult accompanying documents
	Не использовать при нарушении целостности упаковки / Do not use if package damaged
SORB MTP	Планшет / EIA strips
CAL	Калибровочные пробы / Calibrator set
CONTROL	Контрольная сыворотка / Control sera
CONJ HRP	Конъюгат / Conjugate
SUBS TMB	Раствор субстрата тетраметилбензидина (ТМБ) / Substrate solution
BUF WASH 26X	Концентрат отмывочного раствора / Washing solution concentrate
STOP	Стоп-реагент / Stop solution
DIL	ИФА-Буфер / EIA buffer

Уважаемый Клиент!
Если в процессе работы с нашими Наборами Вам понадобились пластиковые ванночки для жидких реагентов, одноразовые наконечники для дозаторов или дополнительные объемы реагентов (концентрат отмывочного раствора, ИФА-Буфер, раствор субстрата тетраметилбензидина (ТМБ), стоп-реагент), входящих в состав Набора, просим Вас обратиться к поставщику продукции ООО «ХЕМА» в Вашем регионе.
Все указанные расходные материалы предоставляются бесплатно, в необходимом для проведения анализа количестве.

Перечень Наборов реагентов для диагностики инфекционных заболеваний производства ООО «ХЕМА»

№ по каталогу	Наименование
K101	«Toxoplasma IgG-ИФА»
K101M	«Toxoplasma IgM-ИФА»
K102	«Rubella IgG-ИФА»
K102M	«Rubella IgM-ИФА»
K103	«Cytomegalovirus IgG-ИФА»
K103M	«Cytomegalovirus IgM-ИФА»
K104	«HSV 1,2 IgG-ИФА»
K104M	«HSV 1,2 IgM-ИФА»
K105	«Chlamydia IgG-ИФА»
K106	«Mycoplasma IgG-ИФА»
K111G	«Сифилис IgG-ИФА»
K111	«Сифилис суммарные антитела-ИФА»
K121	«Aspergillus IgG-ИФА»

Номер горячей линии технической поддержки Клиентов:
8 800 505 23 45
Все звонки на номер горячей линии бесплатны для звонящего с любого мобильного или стационарного телефона по всей территории России.

Ждем Ваших отзывов и предложений по адресам:
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xemahelp

xemahelp@gmail.com

Instruction for use

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ НАБОРА РЕАГЕНТОВ ДЛЯ ИММУНОФЕРМЕНТНОГО ОПРЕДЕЛЕНИЯ ОБЩЕГО IgM В БИОЛОГИЧЕСКИХ ЖИДКОСТЯХ

«Общий IgM-ИФА»

A SOLID-PHASE ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF TOTAL IgM IN HUMAN BIOLOGICAL FLUIDS

Total IgM EIA

НОМЕР ПО КАТАЛОГУ REF **K277**

ТУ № 9398-277-18619450-2009

РЕГИСТРАЦИОННОЕ УДОСТОВЕРЕНИЕ
№ ФСР 2009/06102 от 19 ноября 2009 г.

Антитела к ВИЧ 1,2, вирусу гепатита С и HBsAg отсутствуют
Контрольные сыворотки, входящие в состав набора, инактивированы.

Σ For 96 determinations/На 96 определений

IVD Для *ин витро* диагностики

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105264 Moscow, Russia
Telephone/fax: +7(495) 737-39-36; 737-00-40
e-mail: redkin@xema-medica.com
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CE EC REP

Authorized Representative in EU:
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Steinacker 20, D-73773
Aichwald, Germany
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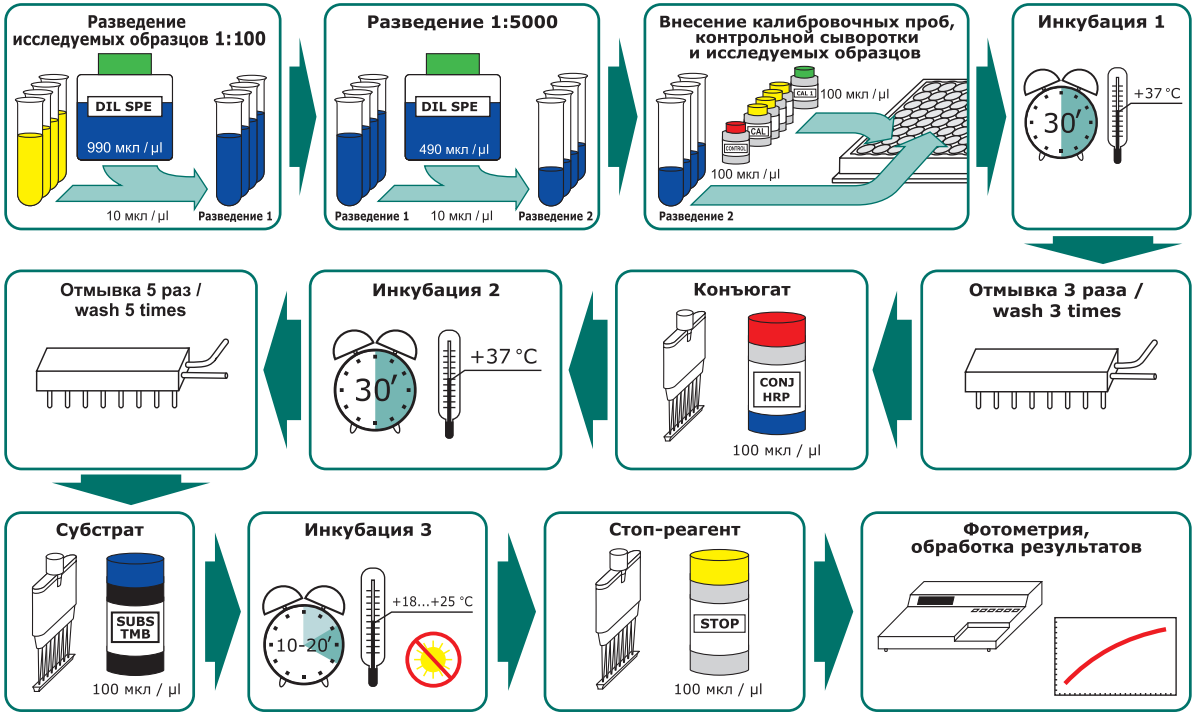
Схема проведения анализа / Test procedure *



K271, K274, K275, K277

* Для сыворотки (плазмы) крови.
Способ разведения для других видов материала приведен в таблице М

* Blood serum or plasma
For other tested materials, see table M.

СОДЕРЖАНИЕ

CONTENT

Инструкция составлена Руководителем службы клиентского сервиса ООО «ХЕМА», к. б. н. Д. С. Кострикиным

«УТВЕРЖДЕНА»
Приказ Росздравнадзора № 9362-Пр/09 от 19 ноября 2009 г.
КРД 68430 от 24.09.2009 г.

ИНСТРУКЦИЯ ПО ПРИМЕНЕНИЮ НАБОРА РЕАГЕНТОВ ДЛЯ ИММУНОФЕРМЕНТНОГО ОПРЕДЕЛЕНИЯ ОБЩЕГО IgM В БИОЛОГИЧЕСКИХ ЖИДКОСТЯХ «Общий IgM-ИФА»

1. НАЗНАЧЕНИЕ

1.1. Набор реагентов «Общий IgM-ИФА» предназначен для количественного определения концентрации общего IgM в биологических жидкостях (см. таблицу М) методом твердофазного иммуноферментного анализа.

1.2. Иммуноглобулин М (IgM) присутствует в крови как в мономерной, так и в пентамерной формах. Повышенная концентрация IgM в сыворотке крови является основным признаком первичного иммунного ответа, а также может свидетельствовать о персистирующих бактериальных и вирусных инфекциях. Снижение концентрации IgM в сыворотке крови наблюдается при некоторых иммунодефицитах. Резкое повышение уровня IgM характерно для макроглобулинемии (болезни Вальденстрема) и IgM миеломы.

2. ПРИНЦИП РАБОТЫ НАБОРА

Определение общего IgM основано на использовании «сэндвич»-варианта твердофазного иммуноферментного анализа. На внутренней поверхности лунок планшета иммобилизованы мышиные моноклональные антитела к общему IgM человека. В лунках планшета, при добавлении исследуемого образца, происходит связывание общего IgM, содержащегося в исследуемом образце, с антителами на твердой фазе. Образовавшийся комплекс выявляют с помощью конъюгата мышиных моноклональных антител к общему IgM с пероксидазой хрена. В результате образуется связанный с пластиком «сэндвич», содержащий пероксидазу. Во время инкубации с раствором субстрата тетраметилбензидина (ТМБ) происходит окрашивание растворов в лунках. Интенсивность окраски прямо пропорциональна концентрации общего IgM в исследуемом образце. Концентрацию общего IgM в исследуемых образцах определяют по калибровочному графику зависимости оптической плотности от содержания общего IgM в калибровочных пробах.

3. АНАЛИТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

3.1. Специфичность. Перекрестная реакция мышиных моноклональных антител к общему IgM с другими аналитами приведена в таблице:

Аналит	Перекрестная реакция, %
IgA	<0.1
IgG	<0.1
IgE	<0.1

3.2. Воспроизводимость.

Коэффициент вариации результатов определения содержания общего IgM в одном и том же образце биологических жидкостей с использованием Набора «Общий IgM-ИФА» не превышает 8.0%.

3.3. Линейность.

Зависимость концентрации общего IgM в образцах биологических жидкостей при разведении их биологическими жидкостями, не содержащей общий IgM, имеет линейный характер в диапазоне концентраций 0.5–10 г/л и составляет ±10.0%.

3.4. Точность.

Данный аналитический параметр проверяется тестом на «открытие» – соответствие измеренной концентрации общего IgM предписанной, полученной путем смешивания равных объемов контрольной сыворотки и калибровочной пробы 2.0 г/л. Процент «открытия» составляет 90–110%.

3.5. Чувствительность.

Минимальная достоверно определяемая Набором «Общий IgM-ИФА» концентрация общего IgM в биологических жидкостях не превышает 0.06 г/л.

4. СОСТАВ НАБОРА

	Код компонента	Символ	Наименование	Кол-во	Ед.	Описание
1	P277Z	SORB MTP	**Планшет** 96-луночный полистироловый, стрипированный, готов к использованию	1	шт.	-
2	C277Z	CAL 1–5	**Калибровочные пробы** на основе трис-буфера (pH 7.2–7.4), содержащие известные количества общего IgM – **0**; **0.5**; **2**; **5**; **10 г/л**, готовы к использованию (по 1 мл каждая)	5	шт.	прозрачные жидкости пурпурного цвета (калибровочная проба 0 – прозрачная бесцветная жидкость)
3	Q277Z	CONTROL	**Контрольная сыворотка** на основе сыворотки крови человека с известным содержанием общего IgM, готова к использованию (1 мл)	1	шт.	прозрачная бесцветная жидкость
4	T277Z	CONJ HRP	**Конъюгат**, готов к использованию (14 мл)	1	шт.	прозрачная жидкость пурпурного цвета
5	S011Z4	DIL SPE	**ИФА-Буфер**, готов к использованию (100 мл)	1	шт.	прозрачная жидкость синего цвета
6	R055Z	SUBS TMB	**Раствор субстрата тетраметилбензидина** (ТМБ), готов к использованию (14 мл)	1	шт.	прозрачная бесцветная жидкость
7	S008Z	BUF WASH 26X	**Концентрат отмывочного раствора** (солевой раствор с твин-20 и бензойной кислотой), 26х-кратный (22 мл)	1	шт.	прозрачная бесцветная жидкость
8	R050Z	STOP	**Стоп-реагент**, готов к использованию (14 мл)	1	шт.	прозрачная бесцветная жидкость
9	N003	-	Бумага для заклеивания планшета	2	шт.	-
10	K277I	-	Инструкция по применению Набора реагентов «Общий IgM-ИФА»	1	шт.	-
11	K277Q	-	Паспорт контроля качества Набора реагентов «Общий IgM-ИФА»	1	шт.	-

5. МЕРЫ ПРЕДОСТОРОЖНОСТИ

5.1. Потенциальный риск применения Набора – класс 2а (ГОСТ Р 51609-2000).

5.2. Все компоненты Набора, за исключением стоп-реагента (5.0% раствор серной кислоты), в используемых концентрациях являются нетоксичными.

Раствор серной кислоты обладает раздражающим действием. Избегать разбрызгивания и попадания на кожу и слизистые. При попадании на кожу и слизистые пораженный участок следует промыть большим количеством проточной воды.

5.3. При работе с Набором следует соблюдать «Правила устройства, техники безопасности, производственной санитарии, противоэпидемического режима и личной гигиены при работе в лабораториях (отделениях, отделах) санитарно-эпидемиологических учреждений системы Министерства здравоохранения СССР» (Москва, 1981 г.).

5.4. При работе с Набором следует надевать одноразовые резиновые или пластиковые перчатки, так как образцы крови человека следует рассматривать как потенциально инфицированный материал, способный длительное время сохранять и передавать ВИЧ, вирус гепатита или любой другой возбудитель вирусной инфекции.

6. ОБОРУДОВАНИЕ И МАТЕРИАЛЫ, НЕОБХОДИМЫЕ ПРИ РАБОТЕ С НАБОРОМ

- фотометр вертикального сканирования, позволяющий измерять оптическую плотность содержимого лунок планшета при длине волны 450 нм;
- термостат, поддерживающий температуру +37 °С ±0.1 °С;
- дозаторы со сменными наконечниками, позволяющие отбирать объемы в диапазоне 5–250 мкл;
- цилиндр мерный вместимостью 1000 мл;
- вода дистиллированная;
- перчатки резиновые или пластиковые;
- бумага фильтровальная.

7. ПОДГОТОВКА РЕАГЕНТОВ ДЛЯ АНАЛИЗА

7.1. Перед проведением анализа компоненты Набора и исследуемые образцы сыворотки (плазмы) крови следует выдержать при комнатной температуре (+18...+25 °С) не менее 30 мин.

7.2. Приготовление планшета.

Вскрыть пакет с планшетом и установить на рамку необходимое количество стрипов. Оставшиеся неиспользованными стрипы, чтобы предотвратить воздействие на них влаги, тщательно заклеить бумагой для заклеивания планшета и хранить при температуре +2...+8 °С в течение всего срока годности Набора.

7.3. Приготовление отмывочного раствора.

Содержимое флакона с концентратом отмывочного раствора (22 мл), перенести в мерный цилиндр вместимостью 1000 мл, добавить 550 мл дистиллированной воды и тщательно перемешать. В случае дробного использования Набора следует отобрать необходимое количество концентрата отмывочного раствора и развести дистиллированной водой в 26 раз (1 мл концентрата отмывочного раствора + 25 мл дистиллированной воды).

8. УСЛОВИЯ ХРАНЕНИЯ И ЭКСПЛУАТАЦИИ НАБОРА

8.1. Набор реагентов «Общий IgM-ИФА» должен храниться в упаковке предприятия-изготовителя при температуре +2...+8 °С в течение всего срока годности, указанного на упаковке Набора.

Допускается хранение (транспортировка) Набора при температуре до +25 °С не более 15 суток. Не допускается замораживание целого набора.

8.2. Набор рассчитан на проведение анализа в дубликатах 42 исследуемых образцов, 5 калибровочных проб и 1 пробы контрольной сыворотки (всего 96 определений).

8.3. В случае дробного использования Набора компоненты следует хранить следующим образом:

- оставшиеся неиспользованными стрипы необходимо тщательно заклеить бумагой для заклеивания планшета и хранить при температуре +2...+8 °С в течение всего срока годности Набора;
- Буфер для разведения образцов, конъюгат, субстрат, стоп-реагент после вскрытия флаконов следует хранить при температуре +2...+8 °С в течение всего срока годности Набора;
- калибровочные пробы и контрольную сыворотку после вскрытия флаконов следует хранить при температуре +2...+8 °С не более 2 месяцев;
- оставшийся неиспользованным концентрат отмывочного раствора следует хранить при температуре +2…+8 °С в течение всего срока годности Набора. Приготовленный отмывочный раствор следует хранить при комнатной температуре (+18…+25 °С) не более 15 суток или при температуре +2…+8 °С не более 45 суток.

Примечание. После использования реагента немедленно закрывайте крышку флакона. Закрывайте каждый флакон своей крышкой.

8.4. Для проведения анализа не следует использовать гемолизированную, мутную сыворотку (плазму) крови, а также сыворотку (плазму) крови, содержащую азид натрия. Если анализ производится не в день взятия крови, сыворотку (плазму) следует хранить при температуре -20 °С. Повторное замораживание-оттаивание образцов сыворотки (плазмы) крови не допускается. Допускается исследование сывороток, хранение которых с момента забора крови осуществлялось при температуре от +2 ºС до +8 ºС не более 7 суток.

8.5. Исключается использование для анализа образцов сыворотки (плазмы) крови людей, получавших в целях диагностики или терапии препараты, в состав которых входят мышиные антитела.

8.6. При использовании Набора для проведения нескольких независимых серий анализов следует иметь в виду, что для каждого независимого определения необходимо построение нового калибровочного графика; кроме этого, рекомендуется определение концентрации общего IgM в контрольной сыворотке.

8.7. Для получения надежных результатов необходимо строгое соблюдение Инструкции по применению Набора.

8.8. Не используйте компоненты из других наборов или из аналогичных наборов других серий.

9. ПРОВЕДЕНИЕ АНАЛИЗА

1	Поместите в рамку необходимое количество стрипов – исследуемые образцы в 2 повторах и 12 лунок для калибровочных проб и контрольной сыворотки.
2	**Разбавьте образцы** сыворотки (плазмы) крови в 5000 раз, используя ИФА-Буфер. Пример: в пробирку Разведение 1 (1:100): 10 мкл образца + 990 мкл ИФА-Буфера. В другую пробирку Разведение 2 (1:5000) добавьте 10 мкл Разведения 1 + 490 мкл ИФА-Буфера. Разведение 2 (1:5000) следует использовать в анализе. Способ разведения для других видов материала приведен в таблице М. Не разбавляйте калибровочные пробы и контрольную сыворотку.
3	Если предполагаемая концентрация общего IgM в исследуемом образце превышает 10 г/л, его следует дополнительно развести, используя ИФА-Буфер. Использование других буферов и реагентов для разбавления образцов может искажать результаты определения! Примечание. Для получения надежных результатов рекомендуется использовать несколько последовательных разведений исследуемого образца биологических жидкостей.
4	**Внесите в соответствующие лунки в дубликатах по 100 мкл каждой калибровочной пробы и контрольной сыворотки**. При исследовании сыворотки (плазмы) крови в лунки, предназначенные для исследуемых образцов, **внесите по 100 мкл разбавленных образцов (Разведение 2)**. При исследовании других видов материала объем вносимого исследуемого образца указан в таблице М. Внесение калибровочных проб, контрольной сыворотки и исследуемых образцов необходимо произвести в течение 15 минут.
5	Аккуратно перемешайте содержимое планшета круговыми движениями по горизонтальной поверхности, заклейте планшет бумагой для заклеивания планшета. **Инкубируйте планшет в течение 30 минут при температуре +37 °С.**
6	По окончании инкубации удалите содержимое лунок аспирацией (например, с помощью водоструйного насоса) или декантированием и **отмойте лунки 3 раза**. При каждой отмывке добавьте во все лунки по 250 мкл отмывочного раствора (см. п. 7.3), встряхните планшет круговыми движениями по горизонтальной поверхности с последующей аспирацией или декантированием. Задержка при отмывке (замачивание лунок) не требуется. При каждом декантировании необходимо тщательно удалять остатки жидкости из лунок.
7	**Внесите во все лунки по 100 мкл конъюгата**.
8	Заклейте планшет бумагой для заклеивания планшета и **инкубируйте** его **в течение 30 минут при температуре +37 °С**.
9	По окончании инкубации удалите содержимое лунок и **отмойте лунки 5 раз**.
10	**Внесите во все лунки по 100 мкл раствора субстрата тетраметилбензидина**. Внесение раствора субстрата тетраметилбензидина в лунки необходимо произвести в течение 2–3 мин. **Инкубируйте планшет в темноте при комнатной температуре (+18...+25 °С) в течение 10–20 минут** в зависимости от степени развития синего окрашивания.
11	**Внесите во все лунки** с той же скоростью и в той же последовательности, как и раствор субстрата тетраметилбензидина, **по 100 мкл стоп-реагента**, при этом содержимое лунок окрашивается в ярко-желтый цвет.
12	**Измерьте величину оптической плотности (ОП) содержимого лунок планшета на фотометре** вертикального сканирования **при длине волны 450 нм**. Измерение ОП содержимого лунок планшета необходимо произвести в течение 15 мин после внесения стоп-реагента. Бланк фотометра выставляйте по калибровочной пробе С1.

продолжение таблицы на стр. 8

13	Постройте в линейных координатах калибровочный график: ось абсцисс (x) – концентрация общего IgM в калибровочных пробах (г/л), ось ординат (y) – оптическая плотность калибровочных проб (ОП 450 нм). Для алгоритма обсчета (аппроксимации) калибровочного графика используйте интервальный (кусочно-линейный, «от точки к точке») метод.
14	Определите по калибровочному графику содержание общего IgM в исследуемых образцах. Если исследуемый образец предразводили (см. п.3), умножьте полученный результат на фактор разведения. При анализе различных видов материала необходимо умножить полученные значения на Фактор пересчета, приведенный в таблице М.

Таблица М

Вид материала	Сбор, хранение и обработка материала	Пример разведения	Буфер для разведения образцов в лунку, мкл	Образец в лунку, мкл	Фактор пересчета
сыворотка (плазма) крови	Исследуемые образцы должны быть тщательно отцентрифугированы. Анализ мутных, хилезных и гемолитических образцов может привести к искажению результатов.	Разведение 1 (1:100): 10 мкл образца + 990 мкл Буфера для разведения образцов. В другую пробирку Разведение 2 (1:5000) добавьте 10 мкл Разведение 1 + 490 мкл Буфера для разведения образцов. Разведение 2 (1:5000) следует использовать в анализе	0	100	1
слюна	Исследуемые образцы должны быть тщательно отцентрифугированы. Анализ мутных образцов может привести к искажению результатов.		90	10	0.002
моча	Исследуемые образцы должны быть тщательно отцентрифугированы. Анализ мутных образцов может привести к искажению результатов.		50	50	0.0004
спинно-мозговая жидкость	Исследуемые образцы должны быть тщательно отцентрифугированы. Анализ мутных образцов может привести к искажению результатов.		80	20	0.001

10. ОЖИДАЕМЫЕ ЗНАЧЕНИЯ И НОРМЫ

10.1. Основываясь на результатах исследований, проведенных ООО «ХЕМА», рекомендуем пользоваться нормами, приведенными ниже. Вместе с тем, в соответствии с правилами *GLP* (Хорошей лабораторной практики), каждая лаборатория должна сама определить параметры нормы, характерные для обследуемой популяции.

Примечание. Значения концентраций общего IgM в исследуемых образцах, находящиеся ниже границы чувствительности Набора (0.06 г/л), а также превышающие значение верхней калибровочной пробы (10 г/л) следует приводить в следующей форме: в исследуемом образце X концентрация общего IgM ниже 0.06 г/л или выше 10 г/л.

Исследуемая группа	Единицы, г/л	
	Нижний предел	Верхний предел
Здоровые доноры	0.7	3.7
новорожденные	0.1	0.35
1-3 месяца	0.12	0.9
4-6 месяцев	0.25	1.2
7-12 месяцев	0.35	1.0
1-6 лет	0.55	2.2
7-11 лет	0.65	1.7

11. ЛИТЕРАТУРА

1. Erik J. Wiersma, Cathy Collins, Shafie Fazel, and Marc J. Shulman Structural and Functional Analysis of J Chain-Deficient IgM J. Immunol., Jun 1998; 160: 5979 – 5989.

По вопросам, касающимся качества Набора **«Общий IgM-ИФА»**,
следует обращаться в ООО «ХЕМА» по адресу:
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Руководитель службы клиентского сервиса ООО «ХЕМА»,
к. б. н. Д. С. Кострикин

Instruction for use

A SOLID-PHASE ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF TOTAL IgM IN HUMAN BIOLOGICAL FLUIDS

1. INTENDED USE

A solid-phase enzyme immunoassay for the quantitative determination of total IgM in biological fluids.

This kit is designed for measurement of total IgM in biological fluids. For possibility of use with other sample types, please, refer to Application Notes (on request). The kit contains reagents sufficient for 96 determinations and allows to analyze 42 unknown samples in duplicates.

2. SUMMARY AND EXPLANATION

Immunoglobulin M (IgM) is secreted during primary immune response and exists in monomeric and pentameric forms. Elevated serum IgM is observed in chronic inflammation, macroglobulinemia and IgM myeloma. Decreased IgM level may occur in some immunodeficiency syndromes.

3. PRINCIPLE OF THE TEST

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific murine monoclonal to human total IgM-antibodies. Antigen from the specimen is captured by the antibodies coated onto the microwell surface. Unbound material is removed by washing procedure. Second antibodies – murine monocnoclonal to human total IgM, labelled with peroxidase enzyme, are then added into the microwells. After subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen.

4. WARNINGS AND PRECAUTIONS

4.1. For professional use only.

4.2. This kit is intended for in vitro diagnostic use only.

4.3. INFECTION HAZARD: There is no available test methods that can absolutely assure that Hepatitis B and C viruses, HIV-½, or other infectious agents are not present in the reagents of this kit. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

4.4. Avoid contact with stop solution containing 5.0% H_2SO_4. It may cause skin irritation and burns.

4.5. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents may give false results.

4.6. Do not use the kit beyond the expiration date.

4.7. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microplate readers.

4.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

4.9. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.

4.10. Do not mix reagents from different lots.

4.11. Replace caps on reagents immediately. Do not swap caps.

4.12. Do not pipette reagents by mouth.

4.13. Specimens must not contain any AZIDE compounds – they inhibit activity of peroxidase.

4.14. Material Safety Data Sheet for this product is available upon request directly from XEMA Co., Ltd.

4.15. The Material Safety Data Sheet fit the requirements of EU Guideline 91/155 EC.

5. KIT COMPONENTS

5.1. Contents of the Kit

	Symbol	Description		Qty	Units	Colour code	Stability of opened/diluted components
1	SORB MTP	total IgM EIA strips, 8x12 wells	polystyrene microwells coated with murine monoclonal to human total IgM	1	pcs		until exp.date
2	CAL 1–5	Calibrator set, 1 ml each. The set contains 5 calibrators: 0; 0.5; 2; 5; 10 g/l	human total IgM diluted in tris buffered BSA solution, preservative – 0.01 % Bronidox L, 0.01 % 2-Methyl-4-isothiazolin-3-one-hydrochloride; also contains red dye	5	pcs	purple (C1 – colourless)	2 months
3	CONTROL	Control serum (1 ml)	dilution of preselected human serum, with high content of total IgM with BSA solution; preservative – 0.01 % Bronidox L, 0.01 % 2-Methyl-4-isothiazolin-3-one-hydrochloride, colourless	1	pcs	colourless	2 months
4	CONJ HRP	Conjugate, 14 ml	aqueous solution of murine monocnoclonal to human total IgM coupled with horseradish peroxidase diluted on phosphate buffered solution with casein from bovine milk and detergent (Tween-20), contains 0.1 % phenol as preservative and red dye	1	pcs	purple	until exp. date
5	DIL SPE	EIA buffer 100 ml	phosphate buffered saline with casein from bovine milk and detergent (Tween-20), contains 0.1% phenol as preservative and blue dye	1	pcs	blue	until exp. date
6	SUBS TMB	Substrate solution, 14 ml	ready-to-use single-component tetramethylbenzidine (TMB) solution.	1	pcs	colourless	until exp.date
7	BUF WASH 26X	Washing solution concentrate 26X, 22 ml	aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative	1	pcs	colourless	Concentrate – until exp.date Diluted washing solution – 45 days at 2-8 °C or 15 days at RT
8	STOP	Stop solution, 14 ml	5.0 % vol/vol solution of sulphuric acid	1	pcs	colourless	until exp. date
9	N003	Plate sealing tape		2	pcs		N/A
10	K277I	Instruction total IgM EIA		1	pcs		N/A
11	K277Q	QC data sheet total IgM EIA		1	pcs		N/A

5.2. Equipment and material required but not provided

- Distilled or deionized water;
- Automatic or semiautomatic multichannel micropipettes, 100–250 µl, is useful but not essential;
- Calibrated micropipettes with variable volume, range volume 5–250 µl;
- Dry thermostat for 37 °C ±0.1 °C
- Calibrated microplate photometer with 450 nm wavelength and OD measuring range 0–3.0

5.3. Storage and stability of the Kit

Store the whole kit at +2...+8 °C upon receipt until the expiration date.

After opening the pouch keep unused microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture.

6. SPECIMEN COLLECTION AND STORAGE

This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided.

Specimens may be stored for up to 48 hours at +2...+8 °C before testing.

7. TEST PROCEDURE

7.1. Reagent Preparation

- All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18...+25 °C) before use.
- All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.
- It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.
- Prepare washing solution from the concentrate BUF WASH 26X by 26 dilutions in distilled water.

7.2. Procedural Note:

It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

7.3. Assay flowchart

See the example of calibration graphic in Quality Control data sheet.

7.4. Assay procedure

1	Put the desired number of microstrips into the frame; allocate 12 wells for the calibrators CAL 1–5 and control samples CONTROL and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.
2	Dilute samples using buffer DIL SPE (EIA sample buffer) 5000 fold. See table M for dilution modes and factors for different types of analyzed material. Do not dilute control sample and calibrators.
3	If suggested analyte concentration in the sample exceeds the highest calibrator, additionally dilute this sample accordingly, using DIL SPE (EIA sample buffer). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.
4	Pipet 100 µl of calibrators CAL 1–5 and control samples CONTROL into allocated wells. For testing of blood serum or plasma pipet 100 µl of the unknown diluted sample (DILUTION 2) into the allocated wells. See table M for the volumes of other materials. Pipetting should be made within 3 minutes, to ensure an uniform incubation time for all samples. Carefully mix the contents of the wells by short horizontal rotating of the plate for 5–7 seconds and cover the wells by plate adhesive tape (included into the kit).
5	Incubate 30 minutes at 37 °C.
6	Prepare washing solution by 26X dilution of washing solution concentrate BUF WASH 26X by distilled water. Minimal quantity of washing solution should be 250 µl per well. Wash strips 3 times.
7	Dispense 100 µl of CONJ HRP into the wells.
8	Incubate 30 minutes at 37 °C.
9	Wash the strips 5 times.
10	Dispense 100 µl of SUBS TMB into the wells.
11	Incubate 10–20 minutes at +18...+25 °C.
12	Dispense 100 µl of STOP into the wells.
13	Measure OD (optical density) at 450 nm.
14	Set photometer blank on first calibrator.
15	Apply point-by-point method for data reduction. Use Calculation factor listed in table M to calculate analyte concentration in different material types.

7.5. Sample processing

Material type	Notes on material collection, storage and handling	Sample dilution example	EIA sample buffer into the well, µl	Sample into the well, µl	Calculation factor
blood serum or plasma	Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.	10 µl of sample + 990 µl of diluent = DILUTION 1. 10 µl of DILUTION1 + 490 µl of diluent = DILUTION 2	0	100	1
saliva	Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.		90	10	0.002
urine	Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.		50	50	0.0004
cerebrospinal fluid	Grossly hemolytic, lipemic, or turbid samples should be avoided and should be treated by centrifugation before testing.		80	20	0.001

8. QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state, and local standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications.

9. CALCULATION OF RESULTS

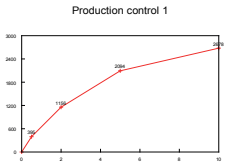
9.1. Calculate the mean absorbance values (OD450) for each pair of calibrators and samples.

9.2. Plot a calibration curve on graph paper: OD versus total IgM concentration.

9.3. Determine the corresponding concentration of total IgM in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

9.4. Below is presented a typical example of a standard curve with the XEMA Co. Not for calculations!

Calibrators	Value	Absorbance Units (450 nm)
CAL 1	0 g/l	0.08
CAL 2	0.5 g/l	0.32
CAL 3	2 g/l	0.93
CAL 4	5 g/l	2.15
CAL 5	10 g/l	3.01



10. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for total IgM. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

Sex, age	Units, g/l	
	Lower limit	Upper limit
Healthy donors	0.7	3.7
newborn	0.1	0.35
1-3 month	0.12	0.9
4-6 month	0.25	1.2
7-12 month	0.35	1.0
1-6 yrs	0.55	2.2
7-11 yrs	0.65	1.7

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical specificity / Cross reactivity

Analyte	Cross-reactivity, % wt/wt
IgA	<0.1
IgG	<0.1
IgE	<0.1

11.2. Analytical sensitivity. Sensitivity of the assay was assessed as being 0.06 g/l.

11.3. Linearity. Linearity was checked by assaying dilution series of 5 samples with different total IgM concentrations. Linearity percentages obtained ranged within 90 to 110%.

11.4. Recovery. Recovery was estimated by assaying 5 mixed samples with known total IgM concentrations. The recovery percentages ranged from 90 to 110%.

12. LITERATURE

Erik J. Wiersma, Cathy Collins, Shafie Fazel, and Marc J. Shulman Structural and Functional Analysis of J Chain-Deficient IgM J. Immunol., Jun 1998; 160: 5979 – 5989.

Символ / Symbol	Значение символа / Symbolize
	Производитель / Manufacturer
	Дата производства / Date of manufacture
REF	Номер по каталогу / Catalogue number
LOT	Номер серии / Batch code
 YYYY-MM	Использовать до (год-месяц) / Use By
	Ограничение температуры / Temperature limitation
IVD	Только для ин витро диагностики / In Vitro Diagnostic Medical Device
	Внимание! / Caution, consult accompanying documents
	Не использовать при нарушении целостности упаковки / Do not use if package damaged
SORB MTP	Планшет / EIA strips
CAL	Калибровочные пробы / Calibrator set
CONTROL	Контрольная сыворотка / Control sera
CONJ HRP	Конъюгат / Conjugate
SUBS TMB	Раствор субстрата тетраметилбензидина (ТМБ) / Substrate solution
BUF WASH 26X	Концентрат отмывочного раствора / Washing solution concentrate
STOP	Стоп-реагент / Stop solution
DIL	ИФА-Буфер / EIA buffer

Уважаемый Клиент!
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Перечень Наборов реагентов для диагностики инфекционных заболеваний производства ООО «ХЕМА»

№ по каталогу	Наименование
K101	«Toxoplasma IgG-ИФА»
K101M	«Toxoplasma IgM-ИФА»
K102	«Rubella IgG-ИФА»
K102M	«Rubella IgM-ИФА»
K103	«Cytomegalovirus IgG-ИФА»
K103M	«Cytomegalovirus IgM-ИФА»
K104	«HSV 1,2 IgG-ИФА»
K104M	«HSV 1,2 IgM-ИФА»
K105	«Chlamydia IgG-ИФА»
K106	«Mycoplasma IgG-ИФА»
K111G	«Сифилис IgG-ИФА»
K111	«Сифилис суммарные антитела-ИФА»
K121	«Aspergillus IgG-ИФА»

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