ТОВ «ХЕМА» код ЄДРПОУ 36038442
Адреса 03179, м. Київ, вул. Академіка Єфремова, 23
Для кореспонденції: 03179, а/с 49
З питань замовлення продукції: 050-422-62-16, 067-422-62-16
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STATEMENT

We, XEMA LLC, as a manufacturer of in vitro diagnostic medical devices, having a registered office at Akademika Yefremova St. 23, Kyiv, Ukraine assign SRL SANMEDICO having a registered office at A. Corobceanu Street 7A, apt. 9, Chişinău MD-2012, Moldova, as authorized representative in correspondence with legislative requirements of the Republic of Moldova.

We declare that the company mentioned above is authorized to register, notify, renew, or modify the registration of medical devices on the territory of the Republic of Moldova.

This Statement shall come into force on the date of its signing. The duration of this Statement is 3 years from the date of signing.

Date: 06.09.2023

Signature:

Director Xema LLC
Oleksandra Zavaliei

UkrMedCert

8О047
Сертифікація
систем менеджменту

CERTIFICATE

on compliance of Quality Management System

Registration Date:
August 02, 2024
No. UA.SM.214-21
Expiry Date: August 01, 2027
First edition: August 04, 2021

THIS IS TO CERTIFY THAT
QUALITY MANAGEMENT SYSTEM CONCERNING

The Design and Development, Manufacture, Storage and Distribution medical devices for in vitro diagnostics

was implemented by: XEMA LLC
at the address: Akademika Yefremova St. 23, Kyiv, Ukraine, 03179

meets the requirements of DSTU EN ISO 13485:2018 (EN ISO 13485:2016, IDT; ISO 13485:2016, IDT); ISO 13485:2016.

Compliance control of the certified quality management system with the requirements of the specified standard is carried out through supervision, the frequency and procedures of which are regulated by the procedures of the conformity assessment body.

The conformity assessment body UKRMEDCERT LLC, address: str. Drahomanova, building 1-A, office 2, Kyiv, 02059, Ukraine, phone: +38-067-595-02-30, https://ukrmedcert.org.ua

Head of CAB **Tetiana SUKHENKO**

The validity of a certificate of compliance can be verified in the online Register https://ukrmedcert.org.ua or by phone +38-067-595-02-30.
The original version of this Certificate is issued in Ukrainian.

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Vertretung und Repräsentanz

Certificate

Of Marketing Authorization of Medical Product

within Germany, the member states of the European Union
and the other states having a contractual agreement with the European Economic Area

Nr. AR/IVD/XEMA LLC/01/2023

Issued on the basis of the Declaration of conformity and registration taking into account account Article 11 of Regulation (EU) 2017/746 (IVDR) on In Vitro Diagnostic, and Medical Device Implementing Act (MPDG)

Ausgestellt auf Grund der Konformitätserklärung und Registrierung unter Berücksichtigung der der Verordnung (EU) 2017/746 (IVDR) über In-vitro-Diagnostika und Medizinprodukterecht-Durchführungsgesetz (MPDG)

Manufacturer / Hersteller	**XEMA LLC** **SRN: UA-MF-000032959** UKRAINE, 03179 KYIV Akademika Yefremova St. 23 qa@xema.com.ua; www.xema.in.ua
Product name / Produkt	**See annex to the Certificate** Siehe Anhang zum Zertifikat
Product Classification: Produktklassifizierung	**In Vitro Diagnostic Medical Devices** In-vitro-Diagnostikum (IVD) Medizinprodukte
Category: Kategorie	**Common/ Other IVD** Sonstige IVD-Produkte
Conformity assessment procedure: Konformitatsbewertungsverfahren:	**EC DECLARATION OF CONFORMITY** **(Annex III, except point 6, Directive 98/79/EC)** **in connection with article 110(3) IVDR** **EU- KONFORMITATSERKLARUNG** (Anhang III, außer Nummer 6, Richtlinie 98/79 / EG) in Verbindung mit Artikel 110 (3) IVDR
State Competent Authority: Staatliche Zuständige Behörde	**BfArM** Federal Institute for Drugs and Medical Devices DMIDS (German Medical Device Information and Database System) **BfArM** Das Bundesinstitut für Arzneimittel und Medizinprodukte DMIDS (Deutsches Medizinprodukte-Informations- und Datenbanksystem)

Date of issue : **2023-03-07**
Das Ausstellungsdatum

Valid to : **2025-05-31**
Gültig bis

Represented in the EC by:

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Valid with the Extract from the database www.dimdi.de (German Medical Device Information and Database System (DMIDS))
Gilt nur mit :Auszug aus der Datenbank www.dimdi.de (Deutsches Medizinprodukte-Informations- und Datenbanksystem (DMIDS))

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Vertretung und Repräsentanz

Annex to the Certificate No.:

Anhang zum Zertifikat Nr.:

AR/IVD/XEMA LLC/01/2023

The following medical devices can be placed on the market in the Federal Republic of Germany, in the member states of the European Economic Community (EEC) and in the other contract states of the agreement about the European Economic Area.

Die folgenden Medizinprodukte in der Bundesrepublik Deutschland, in den Mitgliedsstaaten der Europäischen Wirtschaftsgemeinschaft (EG) und in den Vertragsstaaten der EG in den Verkehr gebracht werden dürfen.

#	Nomenclature term Nomenklaturbezeichnung	Catalog No. Katalog-Nr.	Name of device Produktbezeichnung	DMIDS Registration number Registriernummer
1.	ASPERGILLUS	K021	GalMAg EIA	**DE/CA64/00115824**
2.	HSV IgG	K104	HSV 1/2 IgG EIA	**DE/CA64/00115826**
3.	HSV IgM	K104M	HSV 1, 2 IgM EIA	**DE/CA64/00115833**
4.	HSV 2 IgG	K104B	HSV 2 IgG EIA	**DE/CA64/00115836**
5.	MYCOPLASMA ANTIBODY ASSAYS	K106	Mycoplasma IgG EIA	**DE/CA64/00115837**
6.	SYPHILIS ANTIBODY ASSAYS TOTAL	K111	anti-Treponema pallidum EIA	**DE/CA64/00115839**
7.	SYPHILIS ANTIBODY IGG	K111G	Treponema pallidum IgG EIA	**DE/CA64/00115840**
8.	H. PYLORI ANTIBODY ASSAYS	K119G	Helicobacter pylori IgG EIA	**DE/CA64/00115850**
9.	OTHER OTHER BACTERIOLOGY IMMUNOASSAY	K126	Ureaplasma IgG EIA	**DE/CA64/00115851**
10.	THYROID PEROXIDASE (INCL. MICROSOMAL) ANTIBODIES	K131	aTPO EIA	**DE/CA64/00115852**
11.	THYROGLOBULIN AUTOANTIBODIES	K132	aTG EIA	**DE/CA64/00115853**
12.	MPO ANCA	K133	aMPO EIA	**DE/CA64/00115854**
13.	TISSUE TRANSGLUTAMINASE ANTIBODIES	K160 K161	anti-TGlu IgG EIA anti-TGlu IgA EIA	**DE/CA64/00115855**
14.	GIARDIA LAMBLIA	K171	anti-Giardia lamblia EIA	**DE/CA64/00115856**
15.	OTHER PARASITOLOGY	K174	Ascaris IgG EIA	**DE/CA64/00115857**
16.	ECHINOCOCCUS	K175	Echinococcus IgG EIA	**DE/CA64/00115858**
17.	DISTOMATOSIS	K176	Opisthorchis IgG EIA	**DE/CA64/00115859**
18.	GLIADIN ANTIBODIES	K180 K181	Gliadin IgG EIA Gliadin IgA EIA	**DE/CA64/00115860**
19.	IMMUNOGLOBULIN E – TOTAL	K200	Total IgE EIA	**DE/CA64/00115861**
20.	THYROID STIMULATING HORMONE	K201	TSH EIA	**DE/CA64/00115863**
21.	LUTEINISING HORMONE	K202	LH EIA	**DE/CA64/00115864**
22.	FOLLICLE STIMULATING HORMONE	K203	FSH EIA	**DE/CA64/00115865**
23.	HUMAN GROWTH HORMONE	K204	GH EIA	**DE/CA64/00115866**
24.	HUMAN CHORIONIC GONADOTROPIN TOTAL	K205	hCG EIA	**DE/CA64/00115867**
25.	PROLACTIN	K206	Prolactin EIA	**DE/CA64/00115868**

The above-mentioned medical products are marked with the CE symbol.
Die oben genannten medizinischen Produkte sind mit dem CE-Zeichen gekennzeichnet.

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#	Nomenclature term Nomenklaturbezeichnung	Catalog No. Katalog-Nr.	Name of device Produktbezeichnung	DMIDS Registration number Registriernummer
26.	PROGESTERONE	K207	Progesterone EIA	**DE/CA64/00115869**
27.	ESTRADIOL	K208	Estradiol EIA	**DE/CA64/00115870**
28.	TESTOSTERONE (WITH DEHYDRO AND FREE TESTOSTERONE)	K209	Testosterone EIA	**DE/CA64/00115871**
29.	CORTISOL	K210	Cortisol EIA	**DE/CA64/00115872**
30.	TRIIODOTHYRONINE	K211	T3 EIA	**DE/CA64/00115873**
31.	THYROXINE	K212	T4 EIA	**DE/CA64/00115874**
32.	FREE TRIIODOTHYRONINE	K213	fT3 EIA	**DE/CA64/00115875**
33.	FREE THYROXINE	K214	fT4 EIA	**DE/CA64/00115876**
34.	DEHYDRO-EPIANDROSTERONE SULPHATE (INCL. DHEA)	K215	DHEAS EIA	**DE/CA64/00115877**
35.	17 OH PROGESTERONE	K217	17-OH-progesterone EIA	**DE/CA64/00115878**
36.	ESTRIOL	K218	free Estriol EIA	**DE/CA64/00115880**
37.	TESTOSTERONE (WITH DEHYDRO AND FREE TESTOSTERONE)	K219	free Testosterone EIA	**DE/CA64/00115881**
38.	CANCER ANTIGEN 125	K222	CA 125 EIA	**DE/CA64/00115882**
39.	CANCER ANTIGEN 19-9	K223	CA 19-9 EIA	**DE/CA64/00115883**
40.	CARCINOEMBRYONIC ANTIGEN	K224	CEA EIA	**DE/CA64/00115884**
41.	ALPHAFETOPROTEIN	K225	AFP EIA	**DE/CA64/00115885**
42.	CANCER ANTIGEN 15-3	K226	CA 15-3 (M12) EIA	**DE/CA64/00115886**
43.	OTHER OTHER TUMOUR MARKERS	K232	Thyroglobulin EIA	**DE/CA64/00115887**
44.	ß HUMAN CHORIONIC GONADOTROPIN (INCL. SUBUNIT)	K235	free β-HCG EIA	**DE/CA64/00115888**
45.	CYFRA 21-1	K236	CYFRA 21-1 EIA	**DE/CA64/00115889**
46.	SQUAMOUS CELL CARCINOMA ANTIGEN	K237	SCC (A) EIA	**DE/CA64/00115890**
47.	PREGNANCY ASSOCIATED PLASMA PROTEIN - A (DOWNS)	K238	PAPP-A EIA	**DE/CA64/00115892**
48.	OTHER OTHER TUMOUR MARKERS	K239	HE4 EIA	**DE/CA64/00115893**
49.	CANCER ANTIGEN 242	K243	CA242 EIA	**DE/CA64/00115894**
50.	OTHER PREGNANCY TESTING HORMONES	K245	AMH EIA	**DE/CA64/00115896**

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#	Nomenclature term Nomenklaturbezeichnung	Catalog No. Katalog-Nr.	Name of device Produktbezeichnung	DMIDS Registration number Registriernummer
51.	HUMAN PLACENTAL LACTOGEN HPL	K246	Placental lactogen EIA	DE/CA64/00115897
52.	C-REACTIVE PROTEIN	K250	CRP EIA	DE/CA64/00115898
53.	C-PEPTIDE	K267C	C-peptide EIA	DE/CA64/00115900
54.	INSULIN	K267N	Insulin EIA	DE/CA64/00115901
55.	SEX HORMONE BINDING GLOBULIN	K268	SHBG EIA	DE/CA64/00115902
56.	TROPONIN (T + I)	K291	Troponin I EIA	DE/CA64/00115903
57.	LYME ANTIBODY IGG	K118G	Borelia burgdorferi IgG EIA	DE/CA64/00115904
58.	LYME ANTIBODY IGM	K118M	Borelia burgdorferi IgM EIA	DE/CA64/00115905
59.	EBV ANTIBODIES	K108V K108VM K108N	Epstein-Barr virus VCA IgG EIA Epstein-Barr virus VCA IgM EIA Epstein-Barr virus EBNA IgG EIA	DE/CA64/00115906

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Date: **March 07, 2023**

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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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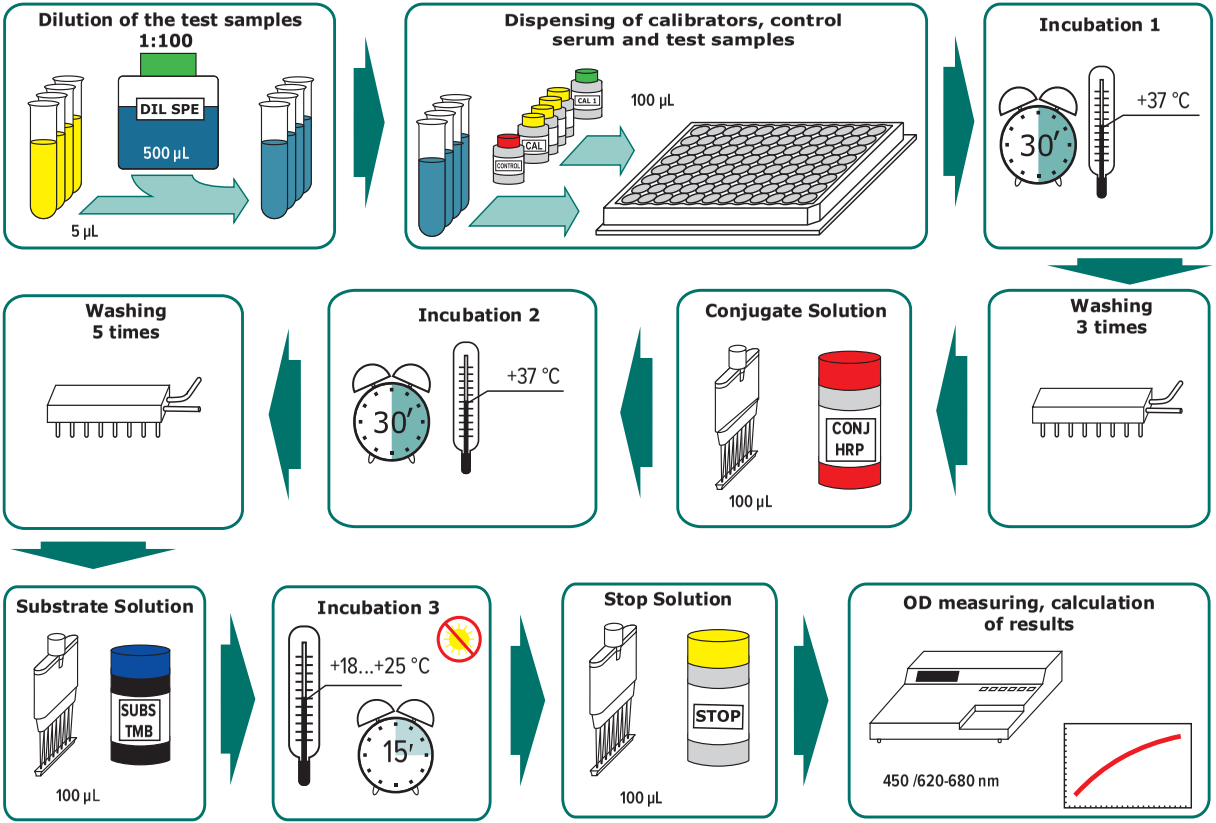
ASSAY PROCEDURE



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.
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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

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SAMPLES IDENTIFICATION PLAN

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	Manufacturer
	*In vitro* diagnistic medical device
	Catalogue number
	Use-by date
	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
	Authorized representative in the European Community/European Union
	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 autoantibodies to thyroglobulin in human serum or plasma

aTG EIA

Catalogue number REF **K132**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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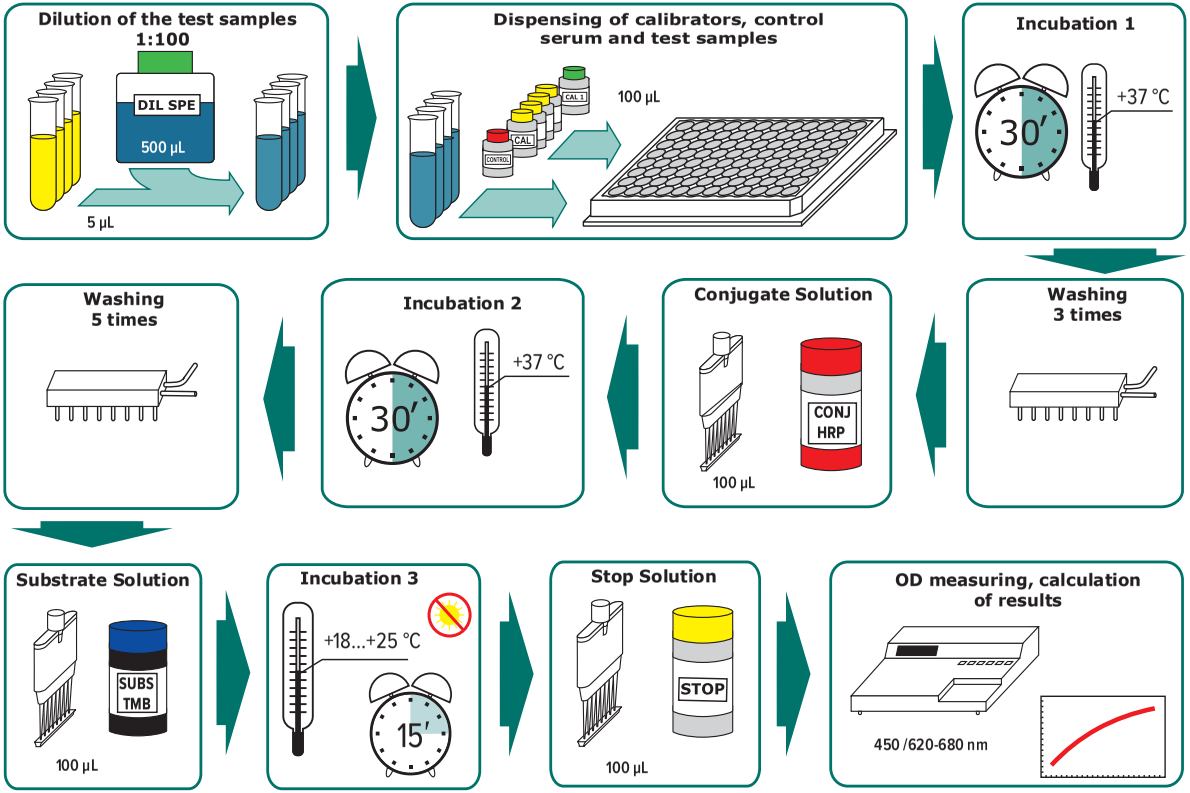
ASSAY PROCEDURE



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.

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 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.
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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».
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SAMPLES IDENTIFICATION PLAN

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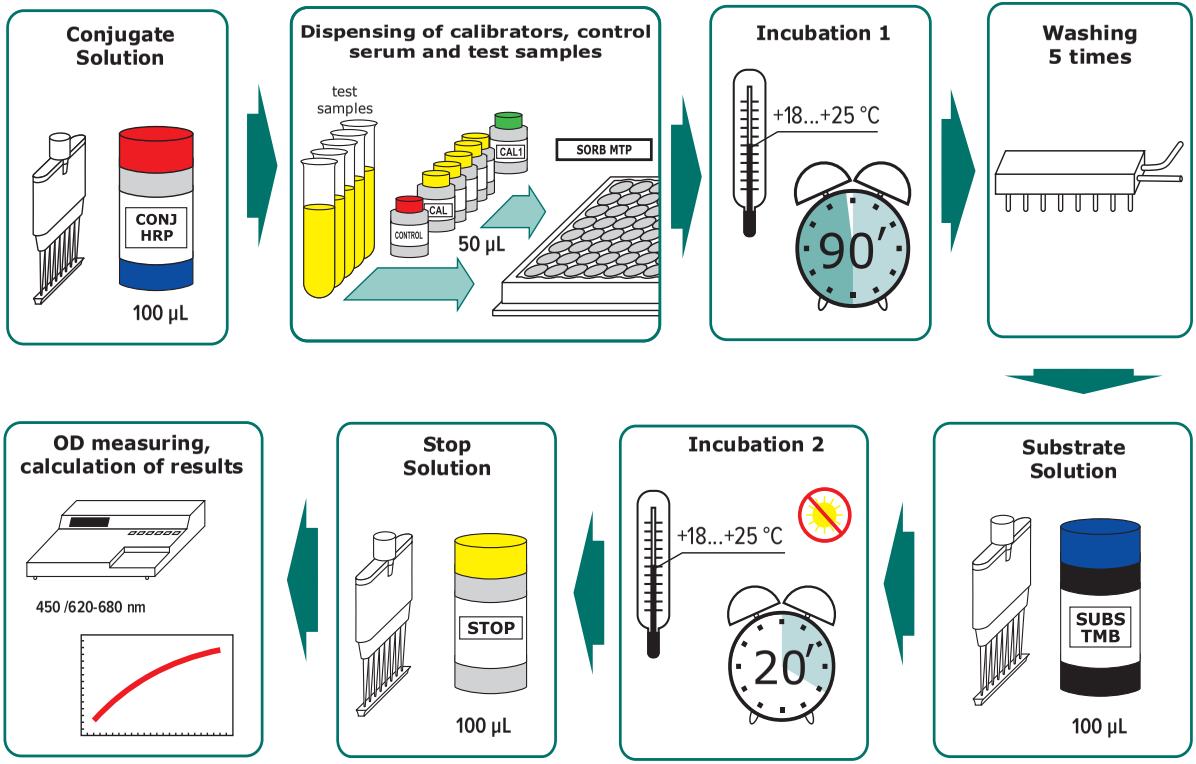
ASSAY PROCEDURE



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

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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
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LOT _____ DATE _____

	Manufacturer
	*In vitro* diagnistic medical device
	Catalogue number
	Use-by date
	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
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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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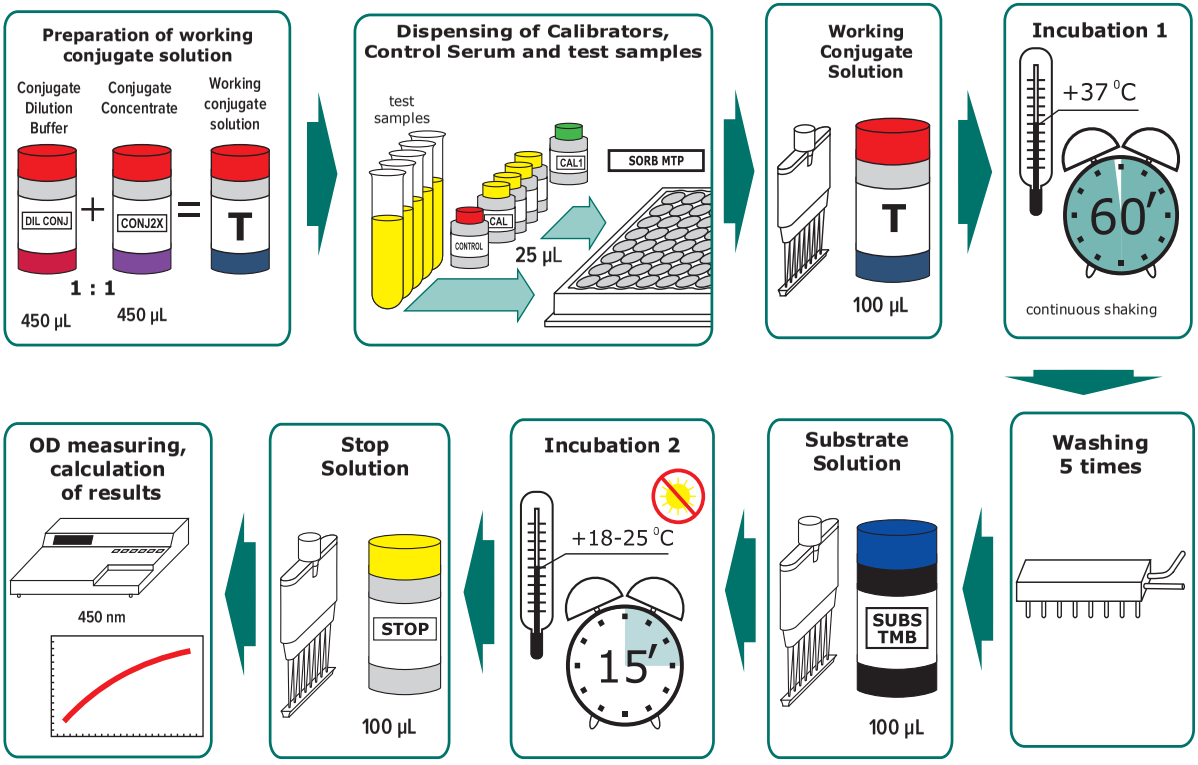
ASSAY PROCEDURE



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.
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».
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
	*In vitro* diagnistic medical device
	Catalogue number
	Use-by date
	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
	Authorized representative in the European Community/European Union
	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 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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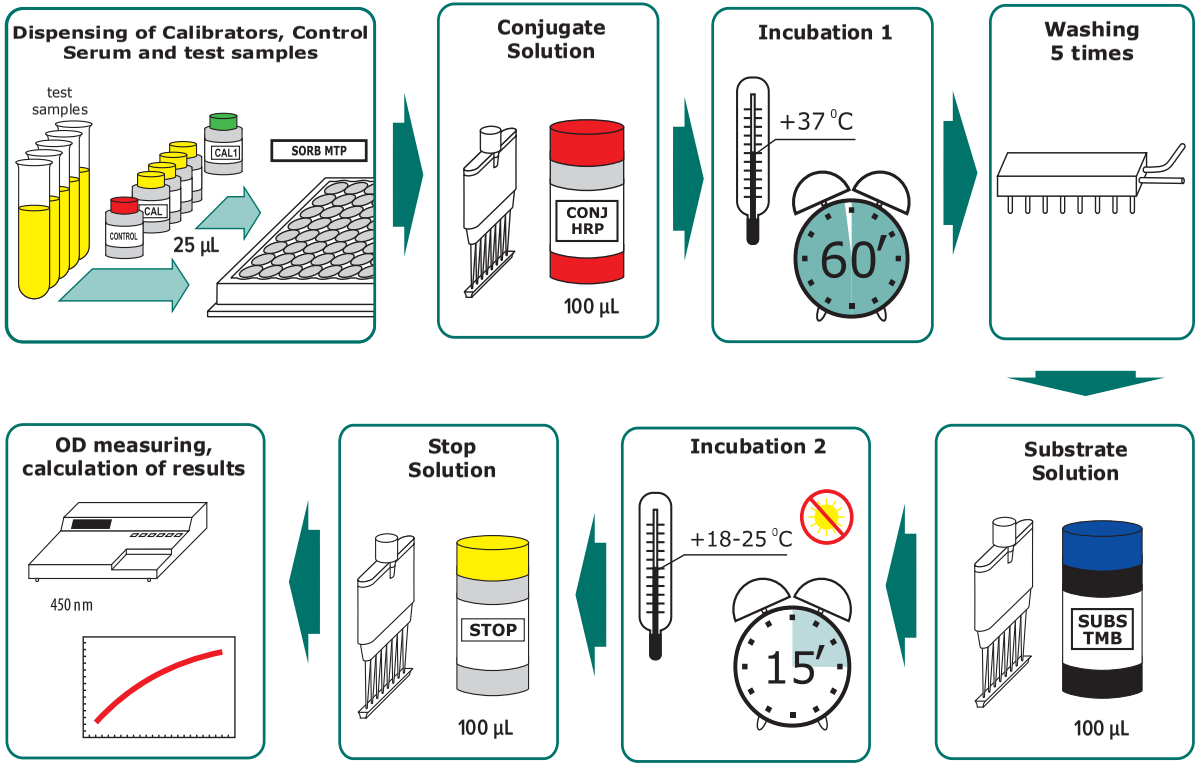
tel.:+ 49 911 931 639 67
E-mail: info@polmed.de
www.polmed.de

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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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												
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D												
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H												

LOT _____ DATE _____

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

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

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