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

ТОВ «УКРМЕДСЕРТ»

СЕРТИФІКАТ

про відповідність системи управління якістю

Зареєстрований у Реєстрі
«29» червня 2022 р.
№ UA.SM.214-21
Дійсний до «03» серпня 2024 р.
Перше видання: «04» серпня 2021 р.

ЦИМ СЕРТИФІКАТОМ ВІДПОВІДНОСТІ ПОСВІДЧУЄТЬСЯ,
ЩО СИСТЕМА УПРАВЛІННЯ ЯКОСТІ СТОСОВНО

проектування та розроблення, виробництва та дистрибуції медичних виробів для діагностики in vitro

впроваджена:

ТОВ «ХЕМА»

за адресою: **вул. Академіка Єфремова, 23, м. Київ, 03179, Україна**

відповідає вимогам ISO 13485:2016;
ДСТУ EN ISO 13485:2018 (EN ISO 13485:2016, IDT; ISO 13485:2016, IDT).

Контроль відповідності сертифікованої системи управління якістю вимогам зазначеного стандарту здійснюється шляхом нагляду, періодичність і процедури якого регламентуються процедурами органу з оцінки відповідності.

Сертифікат видано Органом з оцінки відповідності ТОВ «УКРМЕДСЕРТ», акредитованим Національним агентством з акредитації України, атестат від 24.12.2019 № 8О047, адреса: вул. Драгоманова, будинок 1-А, оф. 2, м. Київ, 02059, Україна, тел./факс: +38-067-595-02-30, https://ukrmedcert.org.ua.

Директор **І.М. Хотенюк**

Чинність сертифіката відповідності можна перевірити в Реєстрі на сайті https://ukrmedcert.org.ua та за тел. +38-067-595-02-30

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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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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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Vertretung und Repräsentanz

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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 pregnancy-associated plasma protein A in human serum or plasma

PAPP-A EIA

Catalogue number REF **K238**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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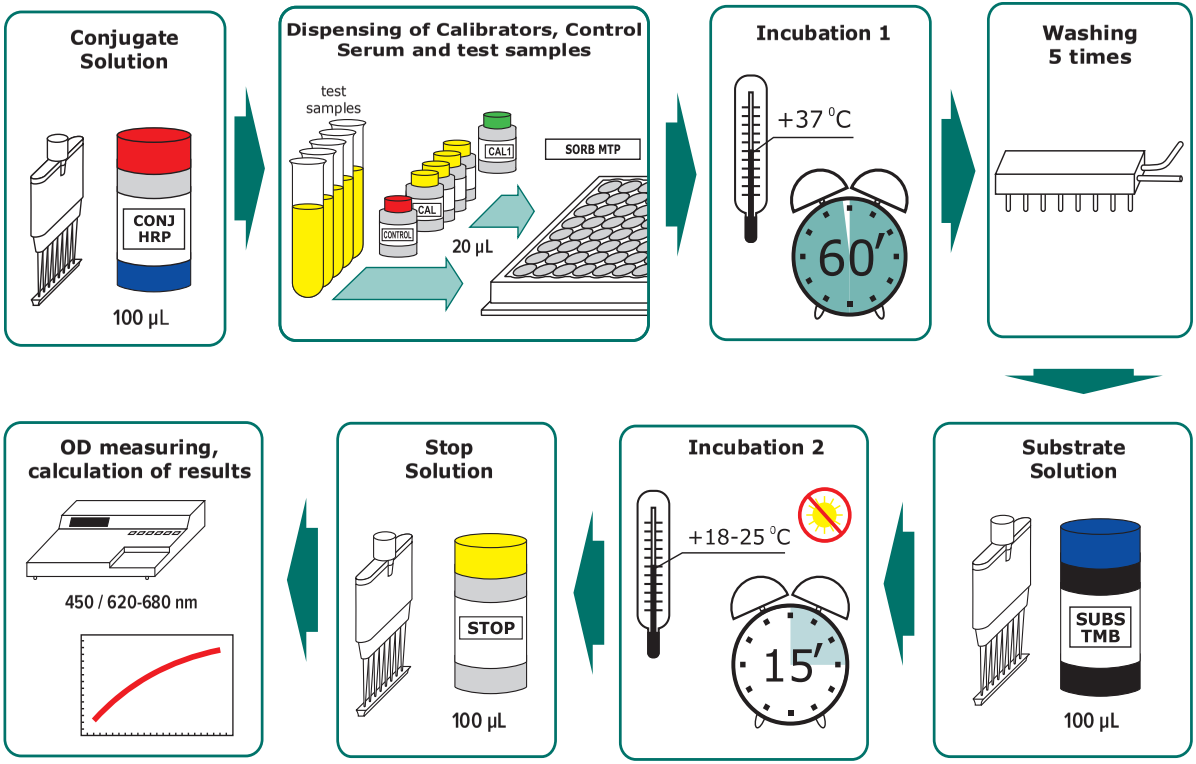
ASSAY PROCEDURE



K238

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of pregnancy-associated plasma protein A in human serum or plasma
PAPP-A EIA

1. INTENDED USE

The PAPP-A EIA kit is an enzyme immunoassay, intended for the quantitative determination of pregnancy-associated plasma protein A in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

PAPP-A (pregnancy-associated plasma protein A) is a high molecular weight glycoprotein consisting of two subunits. In normal pregnancy, PAPP-A level in maternal blood increases during the first two trimesters. Functional significance of PAPP-A during pregnancy remains unclear.

Lowered levels of PAPP-A are observed in Down's syndrome (trisomy 21) during weeks 8-12; after week 14, PAPP-A levels become similar to those in normal pregnancies. Low PAPP-A levels are also found in other trisomies (18 and 13) and chromosomal abnormalities in the fetus and in complicated pregnancies.

Determination of PAPP-A level in the first trimester is used in the following combinations of tests:

- PAPP-A + free beta-HCG.
- PAPP-A + free beta-HCG + USI of nuchal translucency.

In men and non-pregnant women, PAPP-A level is extremely low – usually, it is below the sensitivity level of most immunoassays. Recently, some evidence has appeared to confirm a link between raised PAPP-A levels and increased risk of complications in patients with coronary disease.

3. PRINCIPLE OF THE TEST

The determination of the pregnancy-associated plasma protein A (PAPP-A) is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to human PAPP-A. Second antibodies – murine monoclonal antibodies to human PAPP-A conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

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

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

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

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P238Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to human PAPP-A; ready to use
C238Z	CAL 1	**Calibrator C1**	0.6 mL	1	Solution based on tris buffer (pH 7.2-7.4), free of human PAPP-A, with preservative, ready to use (colourless liquid)
C238Z	CAL 2-6	**Calibrators**	0.6 mL	5	Solution based on tris buffer (pH 7.2-7.4), containing 100; 500; 1000; 5000 and 10000 mU/L of PAPP-A, with preservative, ready to use (blue liquids)
Q238Z	CONTROL	**Control Serum**	0.6 mL	1	Solution based on human serum, containing of known PAPP-A content, with preservative, ready to use (colourless liquid)
T238Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of murine monocnoclonal antibodies to human PAPP-A conjugated to the horseradish peroxidase; ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

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

6. WARNING AND PRECAUTIONS

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

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

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

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

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

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

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

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

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

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

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

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

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

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLE

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

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

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

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

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

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

8.1. Transportation

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

8.2. Storage

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

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

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

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

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

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

8.3. Disposal

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

9. REAGENTS PREPARATION

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

9.2. Microplate preparation

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

9.3. Washing Solution preparation

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

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

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

10. ASSAY PROCEDURE

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

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

10.3 Dispense **20 µL of Calibrators and Control Serum as well as 20 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

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

Scheme of introduction of samples

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

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

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

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

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

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

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

10.10 Determine the corresponding concentration of PAPP-A in tested samples from the calibration curve.

11. TEST VALIDITY

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

12. EXPECTED VALUES

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

12.2. The medians below are based on the analysis of 1840 sera from pregnant women using this kit.

The values shown in the table are for guidance only and can be used to calculate the risk of Down syndrome only if each laboratory has accumulated its own medians. The median values may differ in different geographic areas due to racial and population characteristics.

NOTE: values of PAPP-AP concentrations in the tested samples that are below the LoD (10mU/L) and also exceed the value of the upper Calibrator (10000 mU/L) should be provided in the following form : «the PAPP-A concentration of tested sample X is «lower than 10 mU/L» or «higher than 10000 mU/L».

The calibrators concentration values of the PAPP-A EIA kit are expressed in mU/L. To calculate concentrations in µg/mL, the received concentration value in mU/L shall be multiplied by 0.0045.

1 mU/L = 0.0045 µg/mL

Sex, age	Units, mU/L		Units alternative, µg/mL	
	Lower limit	Upper limit	Lower limit	Upper limit
Males	-	150	-	0.68
Females	-	150	-	0.68

Medians and SD (the recommended range of references is 0.5-2.0 MoM).

Pregnancy, week	Median, mU/L	SD
9	969	2.9
10	1279	3.3
11	2153	3.4
12	3205	3.4
13	4250	3.6

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

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

Sample	Concentration, mU/L	CV, %
1	2319	7.76
2	4879	7.08

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

Sample	Concentration, mU/L	CV, %
1	5342	7.81
2	7853	7.92

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

Sample	Concentration1, mU/L	Concentration2, mU/L	Concentration3, mU/L	CV, %
1	4573	4765	4329	13.6
2	6634	6532	6791	8.1

13.1.2 Trueness

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

13.1.3 Linearity

Linearity was determined using sera samples with known PAPP-A concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 100-1000 mU/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest PAPP-A concentration in the serum or plasma sample that is detected by the PAPP-A EIA kit is no lower than 10 mU/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for PAPP-A EIA kit is 50mU/L.

13.1.5 Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 10000 mU/L.

13.1.6 Analytical specificity

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

14. REFERENCES

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SAMPLES IDENTIFICATION PLAN

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

LOT _____ DATE _____

XEMA

SAMPLES IDENTIFICATION PLAN

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

LOT _____ DATE _____

	Manufacturer
	*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 free β human chorionic gonadotropin in human serum or plasma

free β-HCG EIA

Catalogue number REF **K235**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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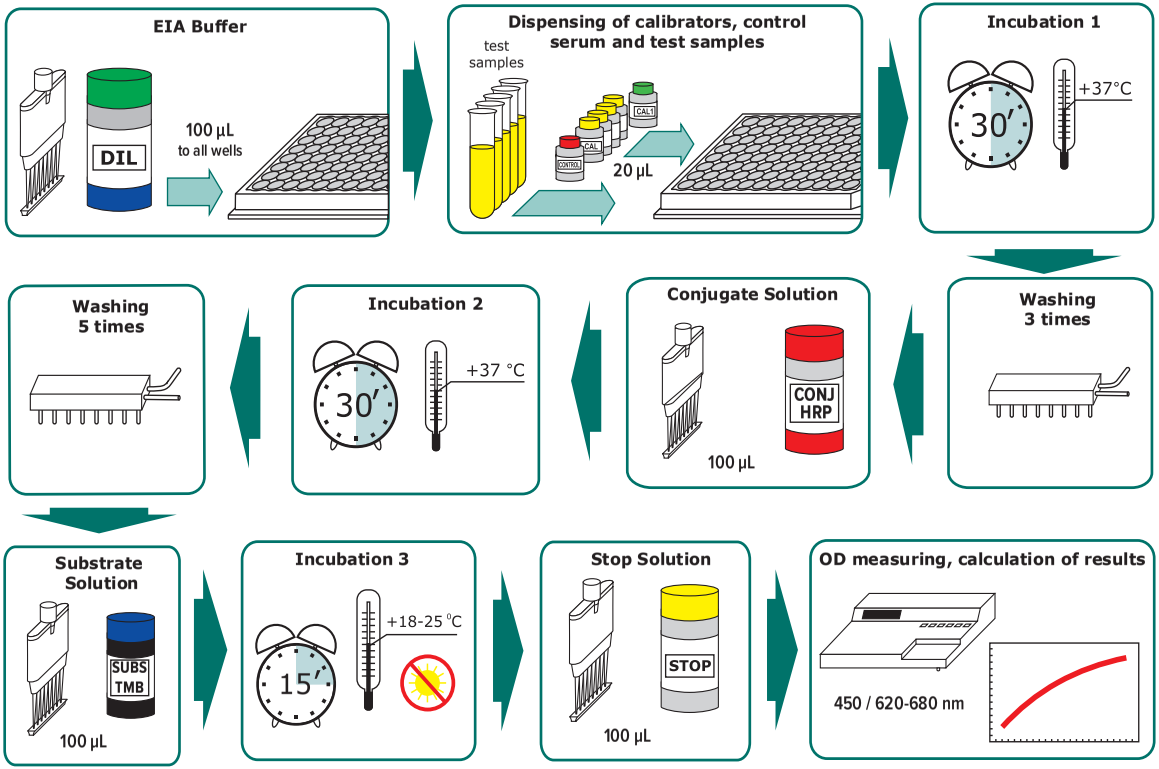
ASSAY PROCEDURE



K235

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SAMPLES IDENTIFICATION PLAN 11

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

1. INTENDED USE

The free β-HCG EIA kit is an enzyme immunoassay, intended for the quantitative determination of free β human chorionic gonadotropin concentration in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Human chorionic gonadotropin (HCG) is a glycoprotein hormone secreted by trophoblastic cells of placenta during pregnancy. HCG appears in blood and urine in about 7-13 day after fertilization, reaching its maximum by the end of the first trimester. An intact molecule of HCG consists of two non-covalently bound polypeptide chains: α- and β-subunits. β-subunit is specific for HCG hormone while α-chain is identical in TSH, LH, FSH and HCG.

Normally, blood levels of free α- and β-chains reach not more than 0.5-1.0% of intact HCG level and during pregnancy vary in parallel with intact HCG. Recently, it was shown that, compared to control, a significant elevation of free β-chain is found in trisomy 21 (Down syndrome), the most pronounced difference being found during weeks 8-9 of pregnancy. That is why determination of free β-chain of HCG in conjunction with other markers (PABB-A, AFP) may be used to estimate risk of congenital pathology of the fetus.

In oncology, a marked rise of free β-chain in blood is found in trophoblastic and germinal tumours (choriocarcinoma, carcinoma of ovaries, etc.).

3. TEST PRINCIPLE

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

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

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

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of free β-HCG in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P235Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to human free β-HCG; ready to use
C235Z	CAL 1	**Calibrator C1**	0.8 mL	1	Solution based on tris buffer (pH 7.2-7.4), free of human free β-HCG, with preservative, ready to use (colourless liquid)
C235Z	CAL 2-5	**Calibrators**	0.8 mL	4	Solutions based on tris buffer (pH 7.2-7.4), containing 10; 50; 120 and 250 ng/mL of human free β-HCG, with preservative, ready to use (green liquids)
Q235Z	CONTROL	**Control Serum**	0.8 mL	1	Solution based on human serum, containing of known human free β-HCG content, with preservative, ready to use (colourless liquid)
T235Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of murine monoclonal antibodies to human free β-HCG conjugated to the horseradish peroxidase; ready to use (purple liquid)
S011Z2	DIL	**EIA Buffer**	22 mL	1	Buffer solution with detergent and preservative, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	2	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (3 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

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

6. WARNING AND PRECAUTIONS

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

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

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

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

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

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

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

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

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

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

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

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

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

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

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

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

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

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

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

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

8.1. Transportation

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

8.2. Storage

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

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

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

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

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

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

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

8.3. Disposal

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

9. REAGENTS PREPARATION

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

9.2. Microplate preparation

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

9.3. Washing solution preparation

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

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

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

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 250 ng/mL, additionally dilute this sample accordingly, using EIA Buffer. Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

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

10. ASSAY PROCEDURE

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

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

10.3 Dispense **100 µL of EIA Buffer** to all wells.

10.4 Dispense **20 µL of Calibrators and Control Serum as well as 20 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

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

Scheme of introduction of samples

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

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

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

10.7 Add **100 µL of the Conjugate Solution** to all wells.

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

10.9 At the end of the incubation period, aspirate and wash each well 5 times as described in 10.6.

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

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

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

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

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

11. TEST VALIDITY

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

12. EXPECTED VALUES

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

NOTE: values of free β-HCG concentrations in the tested samples that are below the LoD (1 ng/mL) and also exceed the value of the upper calibrator (250 ng/mL) should be provided in the following form : «the free β-HCG concentration of tested sample X is «lower than 1 ng/mL» or «higher than 250 ng/mL»

12.2. Expected values and references for the first trimester of pregnancy during calculating the risk of Down syndrome.

The medians below are based on using this kit during analyzing 2108 sera from pregnant women.

The values shown in the table are for guidance only and can be used to calculate the risk of Down syndrome only at the accumulation stage of own medians in each laboratory. The median values may differ depending on geographic areas due to racial and population characteristics.

Pregnancy, week	Median, ng/mL	SD
9	64.3	0.67
10	62	0.62
11	49.2	0.64
12	39.5	0.60
13	39	0.64

12.3. Expected values and references for the second trimester of pregnancy during calculating the risk of Down syndrome.

The data below are based on the analysis of 644 sera from pregnant women in the laboratory of XEMA LLC. Pregnancy dates were determined by the date of the last menstrual period and rounded to the nearest whole month.

The data in the table are for guidance only and are not intended to calculate the risk of Down syndrome.

Pregnancy, week	Units, ng/mL	
	Lower limit	Upper limit
14	21.8	31
15	20.3	28
16	13.3	23
17	11.1	19.9
18	9.9	19.4

Medians and SD (the recommended range of references is 0.5-2.0 MoM).

As new data on medians are accumulated depending on new data - please send your data to control@xema.com.ua.

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

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

Sample	Concentration, ng/mL	CV, %
1	8	1.56
2	232	2.17

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

Sample	Concentration, ng/mL	CV, %
1	8.17	7.33
2	232.7	2.45

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

Sample	Concentration1, ng/mL	Concentration2, ng/mL	Concentration3, ng/mL	CV, %
1	8.14	8.65	8.23	3.26
2	230.4	234.7	232.46	0.92

13.1.2 Trueness

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

13.1.3 Linearity

Linearity was determined using sera samples with known free β-HCG concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 10-250 ng/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest free β-HCG concentration in the serum or plasma sample that is detected by the free β-HCG EIA kit is no lower than 1 ng/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for free β-HCG EIA kit is 10 ng/mL.

13.1.5 Analytical specificity

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

The cross-reactivity of free β-HCG with other analytes is shown in the table:

Analyte	Cross-reactivity, %
LH	< 1
FSH	< 0.2
Prolactin	< 0.5

14. REFERENCES

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7. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

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SAMPLES IDENTIFICATION PLAN

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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
	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:

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

free Testosterone EIA

Catalogue number REF **K219**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

CE

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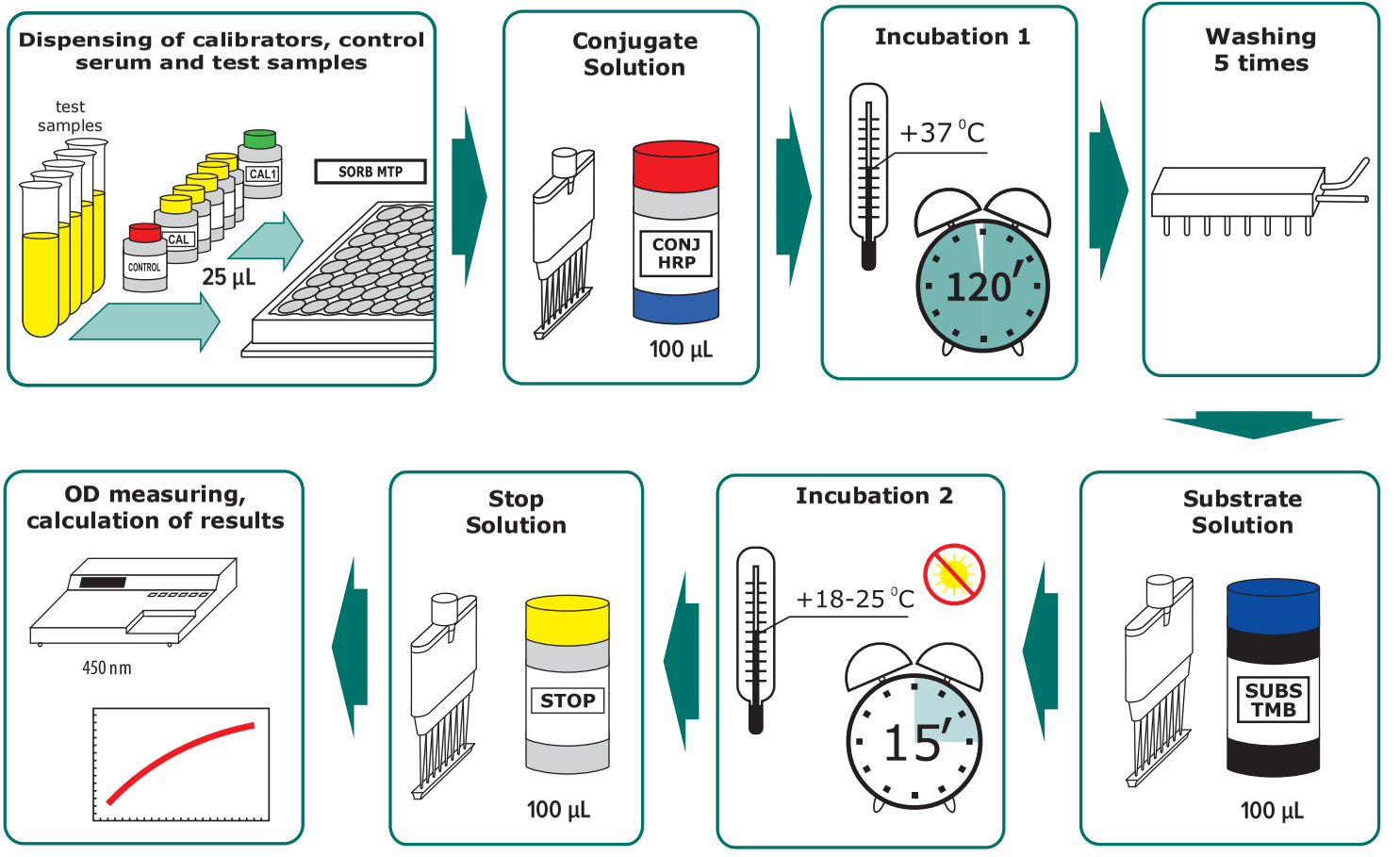
ASSAY PROCEDURE



K219

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SAMPLES IDENTIFICATION PLAN 10

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

1. INTENDED USE

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

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Testosterone is a steroid with a MW of 288.4 Dalton. The main sites of testosterone secretion are Leidig cells in interstitial tissue of testicles in men. In women testosterone is secreted in the adrenals and is controlled by luteinizing hormone. Testosterone stimulates development of male genital organs and formation of secondary sexual features.

In males, testosterone secretion undergoes circadian rhythms with maximal concentrations seen in the morning (6 am) and minimal – in the evening (8 pm). In females, testosterone secretion is regulated by menstrual cycle with maximal levels found in luteinic phase and during ovulation.

Free testosterone is a fraction of serum testosterone not bound to sex-binding globulin hormones (SHBG) and with albumin. Free testosterone makes up 2 - 3% of total testosterone.

Biologically active is only testosterone is free and bound to albumin («bioavailable testosterone»). The level of «bioavailable testosterone» reflects the amount functionally active testosterone in the body.

3. TEST PRINCIPLE

Determination of the free testosterone is based on competition principle of the enzyme immunoassay. Microwells plate is coated with specific to free testosterone murine monoclonal antibodies. Testosterone conjugated to the horseradish peroxidase is used as enzyme conjugate.

The analysis procedure includes two stages of incubation:

- during the first stage free testosterone from the specimen competes with the conjugated testosterone for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed.
- during the second stage, the complexes formed due the reaction with the chromogen 3,3’,5,5’-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. Optical density in the microwell is inversely related to the quantity of the measured free testosterone in the specimen of the serum (plasma).

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

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P219Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to free testosterone, ready to use
C219Z	CAL 1	**Calibrator C1**	0,5 mL	1	Solution based on human plasma, free of testosterone, with preservative, ready to use (yellow liquid)
C219Z	CAL 2-6	**Calibrators**	0,5 mL	5	Solutions based on human plasma, containing 0.2; 1; 4; 20 and 100 pg/mL of free testosterone, with preservative, ready to use (red liquids)
Q219Z	CONTROL	**Control serum**	0,5 mL	1	Solution based on human plasma, containing of known free testosterone content, with preservative, ready to use (colourless liquid)
T219Z	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of testosterone conjugated to the horseradish peroxidase, ready to use (blue liquid
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution, ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid, ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (1 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

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

6. WARNING AND PRECAUTIONS

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

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

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

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

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

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

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

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

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

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

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

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

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

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

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

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

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

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

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

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

8.1. Transportation

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

8.2. Storage

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

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

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

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

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

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

- diluted washing solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

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

8.3. Disposal

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

9. REAGENTS PREPARATION

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

9.2. Microplate preparation

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

9.3. Washing Solution preparation

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

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

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

10. ASSAY PROCEDURE

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

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

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

Scheme of introduction of samples

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

10.3. Add **100 µL of the Conjugate Solution** to all wells.

10.4. Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **120 minutes at +37°C**.

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

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

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

10.8. Read the optical density (OD) of the wells at 450nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.9. Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the free testosterone concentration in the Calibrators pg/mL, (y) – OD versus free testosterone concentration (OD 450 nm). Manual or computerized data reduction is applicable at this stage. For the algorithm calculation (approximation) of the calibration curve, using the interval (segment-linear, point-to-point) method is recommended. Adjust the concentration of CAL1 to an infinitesimally small value, for example, 0.001 pg/mL.

10.10. Determine the corresponding concentration of free testosterone in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 1.2, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

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

NOTE: values of free testosterone concentrations in the tested samples that are below the LoD (0.06 pg/mL) and also exceed the value of the upper calibrator (100 pg/mL) should be provided in the following form: «the free testosterone concentration of tested sample X is «lower than 0.06 pg/mL» or «higher than 100 pg/mL» .

Sex, age	Units, pg/mL	
	Lower limit	Upper limit
Males	4.5	42
Females	-	4.1
Females post menopausal	0.1	4.7

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

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

Sample	Concentration, pg/mL	CV, %
1	10.4	3.46
2	5.6	4.39

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

Sample	Concentration, pg/mL	CV, %
1	10.2	2.33
2	5.1	7.43

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

Sample	Concentration1, pg/mL	Concentration2, pg/mL	Concentration3, pg/mL	CV, %
1	10.5	10.8	10.6	1.44
2	5.4	5.5	5.7	2.76

13.1.2 Trueness

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

13.1.3 Linearity

Linearity was determined using sera samples with known free testosterone concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 0.2-100 pg/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest free testosterone concentration in the serum or plasma sample that is detected by the free Testosterone EIA kit is no lower than 0.06 pg/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for free Testosterone EIA kit is 0.2 pg/mL.

13.1.5 Analytical specificity

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

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

Analyte	Cross-reactivity, %
5-alpha-dehydrotestosterone	16
Androstendiol	1.0
Androstendione	0.4
Androstendione	< 0.1
Dehydroepiandrosterone	< 0.1
Progesterone	< 0.1
Estradiol, Estriol	< 0.01
Cortisol,Pregnenolone	< 0.01

14. REFERENCES

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

free Estriol EIA

Catalogue number REF **K218**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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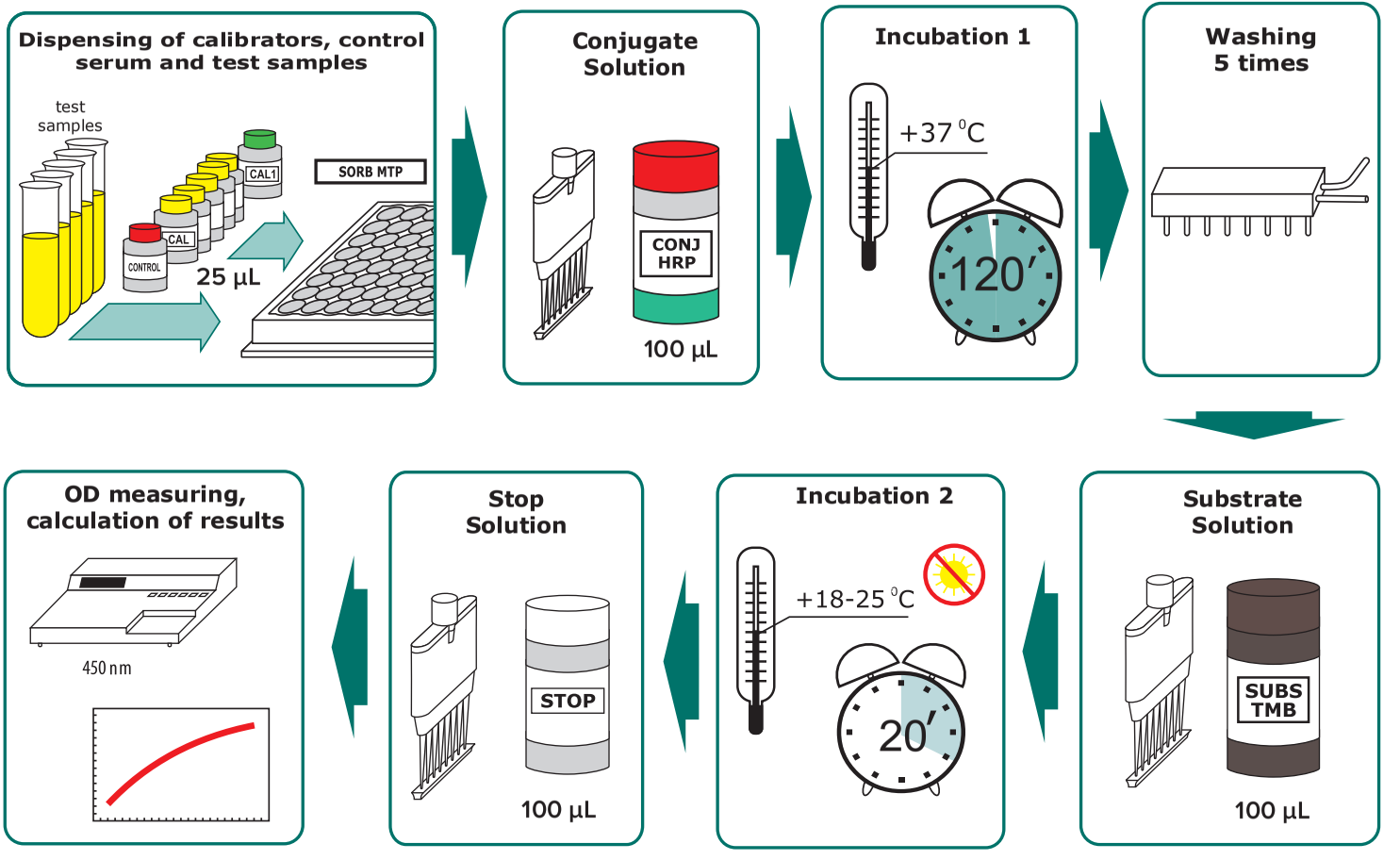
ASSAY PROCEDURE



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SAMPLES IDENTIFICATION PLAN 11

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

free Estriol EIA

1. INTENDED USE

A solid-phase enzyme immunoassay for the quantitative determination of free estriol in blood serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Free (non-conjugated) Estriol (E3) is a steroid hormone with a MM of 288 Da. Production of Estriol in non-pregnant women and men is negligible with no sex-related differences. During pregnancy, Estriol is produced in placenta by a multistep metabolic pathway, and its serum level is continuously growing up to delivery. Due to wide normal ranges of Free E3 at different pregnancy terms, serial determinations to monitor Estriol level are recommended.

Estriol biological function is to maintain blood flow in maternal blood vessels and to provide for differentiation of mammary ducts.

During 2nd trimester, E3 determination in conjunction with other tests (AFP, beta- HCG) is used to estimate risk of Down syndrome in fetus: a tendency of serum E3 level to decline is indicative of Down syndrome. Besides, a decline of E3 serum level during the 2nd trimester may indicate dangerous feto-placental insufficiency (an increased risk of miscarriage, intra-uterine infections, etc.).

Increased E3 levels are seen in multifetal pregnancy and in case of a fetus bigger than normal.

3. TEST PRINCIPLE

Determination of the free Estriol is based on competition principle of the enzyme immunoassay. Microwells plate is coated with the polyclonal rabbit antibodies to the free Estriol. free Estriol conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage free Estriol from the specimen competes with the conjugated free Estriol for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed.
- during the second stage, the complexes formed due the reaction with the chromogen 3,3’,5,5’-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. Optical density in the microwell is inversely related to the quantity of the measured free Estriol in the specimen of the serum (plasma).

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

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P218Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with polyclonal rabbit antibodies to the free Estriol; ready to use
C218Z	CAL 1	**Calibrator C1**	0.5 mL	1	Solution based on human plasma, free of free Estriol, with preservative, ready to use (colourless liquid)
C218Z	CAL 2-6	**Calibrators**	0.5 mL	5	Solutions based on human plasma, containing 0.5; 1.5; 5; 15 and 50 nmol/L of free Estriol, with preservative, ready to use (blue liquids)
Q218Z	CONTROL	**Control Serum**	0.5 mL	1	Solution based on human plasma, containing of known free Estriol content, with preservative, ready to use (colourless liquid)
T218Z	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of free Estriol conjugated to the horseradish peroxidase; ready to use (green liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (1 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

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

6. WARNING AND PRECAUTIONS

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

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

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

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

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

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

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

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

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

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

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

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

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

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

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

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

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

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

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

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

8.1. Transportation

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

8.2. Storage

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

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

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

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

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

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

8.3. Disposal

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

9. REAGENTS PREPARATION

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

9.2. Microplate preparation

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

9.3. Washing Solution preparation

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

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

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

10. ASSAY PROCEDURE

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

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

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

Scheme of introduction of samples

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

10.3. Add **100 µL of the Conjugate Solution** to all wells.

10.4. Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **120 minutes at +37°C**.

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 20 minutes**.

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

10.8. Read the optical density (OD) of the wells at 450nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.9. Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the free Estriol concentration in the calibrators nmol/L, (y) – OD versus free Estriol concentration (OD 450 nm). Manual or computerized data reduction is applicable at this stage. For the algorithm calculation (approximation) of the calibration curve, using the interval (segment-linear, point-to-point) method is recommended. Adjust the concentration of CAL1 to an infinitesimally small value, for example, 0.001 nmol/L.

10.10. Determine the corresponding concentration of free Estriol in tested samples from the calibration curve.

11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 1.2, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

12. EXPECTED VALUES

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

NOTE: values of free Estriol concentrations in the tested samples that are below the LoD (0.12 nmol/L) and also exceed the value of the upper calibrator (50 nmol/L) should be provided in the following form : «the free Estriol concentration of tested sample X is «lower than 0.12 nmol/L» or «higher than 50 nmol/L».

12.2. The calibrators concentration values of the free Estriol EIA kit are expressed in nmol/L. To calculate concentrations in ng/mL, the received concentration value in nmol/L shall be multiplied by 0.288.

1 nmol/L = 0.288 ng/mL

Sex, age	Units, nmol/L		Units alternative, ng/mL	
	Lower limit	Upper limit	Lower limit	Upper limit
Males	-	1.0	-	0.29
Females	-	1.5	-	0.43
Pregnancy				
22-23	5.0	20	1.4	5.8
24-25	5.5	24.5	1.6	7.1
26-27	6.3	27.9	1.8	8.0
28-29	6.4	31.0	1.8	8.9
30-32	7.3	32.9	2.1	9
33-34	9.9	40.3	2.9	11.6
35-36	12.2	>50	3.5	>14.4
37-38	15.5	>50	4.5	>14.4
39-40	16.5	>50	4.8	>14.4
40-42	17.5	>50	5.0	>14.4

Median and SKO (recomended norms of 0.5-2.0 MOM)

Pregnancy, week	Median, nmol/L	SKO
14	3.8	0.51
15	5.3	0.76
16	6.2	0.85
17	7.3	0.94
18	9.9	1.05
19	10.7	1.10
20	11.9	1.08
21	13.2	1.08

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 lot of ELISA kit.

Sample	Concentration, nmol/L	CV, %
1	32.11	4.2
2	6.73	6.9

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

Sample	Concentration, nmol/L	CV, %
1	29.66	3.9
2	6.69	6.3

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	13.73	13.34	14.86	5.8
2	8.39	9.01	9.14	9.5

13.1.2. Trueness

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

13.1.3. Linearity

Linearity was determined using sera samples with known free Estriol 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 0.5–50 nmol/L ±10%.

13.1.4. Analytical sensitivity

Limit of detection (LoD) – the lowest free Estriol concentration in the serum or plasma sample that is detected by the free Estriol EIA kit is no lower than 0.12 nmol/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for free Estriol EIA kit is 0.5 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 free Estriol with other analytes is shown in the table:

Analyte	Cross-reactivity, %
Estradiol	0.8
Estrone	0.3
16-epiestriol	1.9
Corticosterone	< 0.1

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												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

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

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

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