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
Тел.: +38 (095) 60-99-555 Факс: +38 (044) 422-62-16
e-mail:info@xema.com.ua
www.xema.in.ua

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

Digitally signed by Dolgopol Iulian
Date: 2024.12.18 12:51:54 EET
Reason: MoldSign Signature
Location: Moldova
MOLDOVA EUROPEANĂ

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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Beata Rozwadowska * Polmed.de * Fichtenstr. 12a, 90763 Fürth, Germany

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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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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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Beata Rozwadowska * Polmed.de * Fichtenstr. 12a, 90763 Fürth, Germany

Date: **March 07, 2023**

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ТОВ «ХЕМА» код ЄДРПОУ 36038442
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ДЕКЛАРАЦІЯ ПРО ВІДПОВІДНІСТЬ № 2 (РЕДАКЦІЯ 7)

1. Назва та номер за каталогом медичного виробу для діагностики in vitro	«Реагенти імуноферментні для діагностики *in vitro*» ТУ У 24.4-36038442-001:2011 згідно з переліком виробів викладеного у додатку до Декларації про відповідність повне найменування медичного(-их) виробу(-ів) для діагностики in vitro, тип, марка, модель
2. Класифікація згідно з Технічним регламентом щодо медичних виробів для діагностики in vitro (Постанова КМУ № 754 від 2 жовтня 2013 р.)	**перелік В Додатка 2**
3. Виробник:	**ТОВ «ХЕМА», вул. Академіка Єфремова, 23, м. Київ, Україна, код за ЄДРПОУ: 36038442 тел./факс (044) 422-62-16, e-mail: info@xema.com.ua** найменування та місцезнаходження, телефон, електронна адреса, код за ЄДРПОУ
4. Процедура оцінки відповідності	Додаток 4 до Технічного регламенту щодо медичних виробів для діагностики in vitro (Постанова КМУ № 754 від 2 жовтня 2013 р.)
5. Призначений орган з оцінки відповідності	ТОВ «УКРМЕДСЕРТ» ідентифікаційний номер UA.TR.099
6. Сертифікати	Сертифікат про відповідність вимогам Технічного регламенту щодо медичних виробів для діагностики in vitro № UA.MD.408-21. Дійсний до 03.08.2026р.

Декларуємо про виконання основних вимог до медичних виробів для діагностики in vitro, наведених у Додатку 1 Технічного регламенту щодо медичних виробів для діагностики in vitro (Постанова КМУ № 754 від 2 жовтня 2013 р.).

Декларація про відповідність складена та видана під виключну відповідальність виробника.

м. Київ	*05.08.2021 р.*	*03.08.2026 р.*
(місце видачі)	(дата складання декларації)	(термін дії декларації до)

Директор ТОВ «ХЕМА» (підпис) **О.О. Завалєй**

Україна * Товариство з обмеженою відповідальністю «ХЕМА» Ідентифікаційний код 36038442

Декларація складена у відповідності до ДСТУ ISO/IEC 17050-1:2006

ДОДАТОК
до декларації про відповідність №2

Перелік виробів, на які розповсюджується декларація про відповідність №2

№ з/п	Найменування продукції	Скорочена назва	Номер за каталогом	Номер за класифікатором медичних виробів НК 024:2019
1	Набір реагентів для імуноферментного визначення IgG антитіл до антигенів *Toxoplasma gondii* в сироватці (плазмі) крові	«Toxoplasma gondii IgG - ІФА»	К101	52436
2	Набір реагентів для імуноферментного виявлення IgM антитіл до антигенів *Toxoplasma gondii* в сироватці (плазмі) крові	«Toxoplasma gondii IgM – ІФА»	К101М	52440
3	Набір реагентів для імуноферментного визначення IgG антитіл до антигенів Rubella в сироватці (плазмі) крові	«Rubella IgG – ІФА»	К102	50265
4	Набір реагентів для імуноферментного виявлення IgM антитіл до антигенів Rubella в сироватці (плазмі) крові	«Rubella IgM – ІФА»	К102М	50268
5	Набір реагентів для імуноферментного визначення IgG антитіл до антигенів Cytomegalovirus в сироватці (плазмі) крові	«Cytomegalovirus IgG – ІФА»	К103	49712
6	Набір реагентів для імуноферментного виявлення IgM антитіл до антигенів Cytomegalovirus в сироватці (плазмі) крові	«Cytomegalovirus IgM-ІФА»	К103М	49723
7	Набір реагентів для імуноферментного виявлення IgG антитіл до антигенів *Chlamydia spp.* в сироватці (плазмі) крові	«Chlamydia IgG-ІФА»	К105	30680
8	Набір реагентів для імуноферментного виявлення IgM антитіл до антигенів *Chlamydia spp.* в сироватці (плазмі) крові	«Chlamydia IgM-ІФА»	К105М	30680
9	Набір реагентів для імуноферментного визначення загального простатичного специфічного антигену в сироватці (плазмі) крові	«Загальний ПСА – ІФА»	К221	54664
10	Набір реагентів для імуноферментного визначення вільного простатичного специфічного антигену в сироватці (плазмі) крові	«Вільний ПСА - ІФА»	К231	54668

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

Cytomegalovirus IgG EIA

ATTENTION! The instruction has been changed!

Catalogue number REF **K103**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

XEMA LLC
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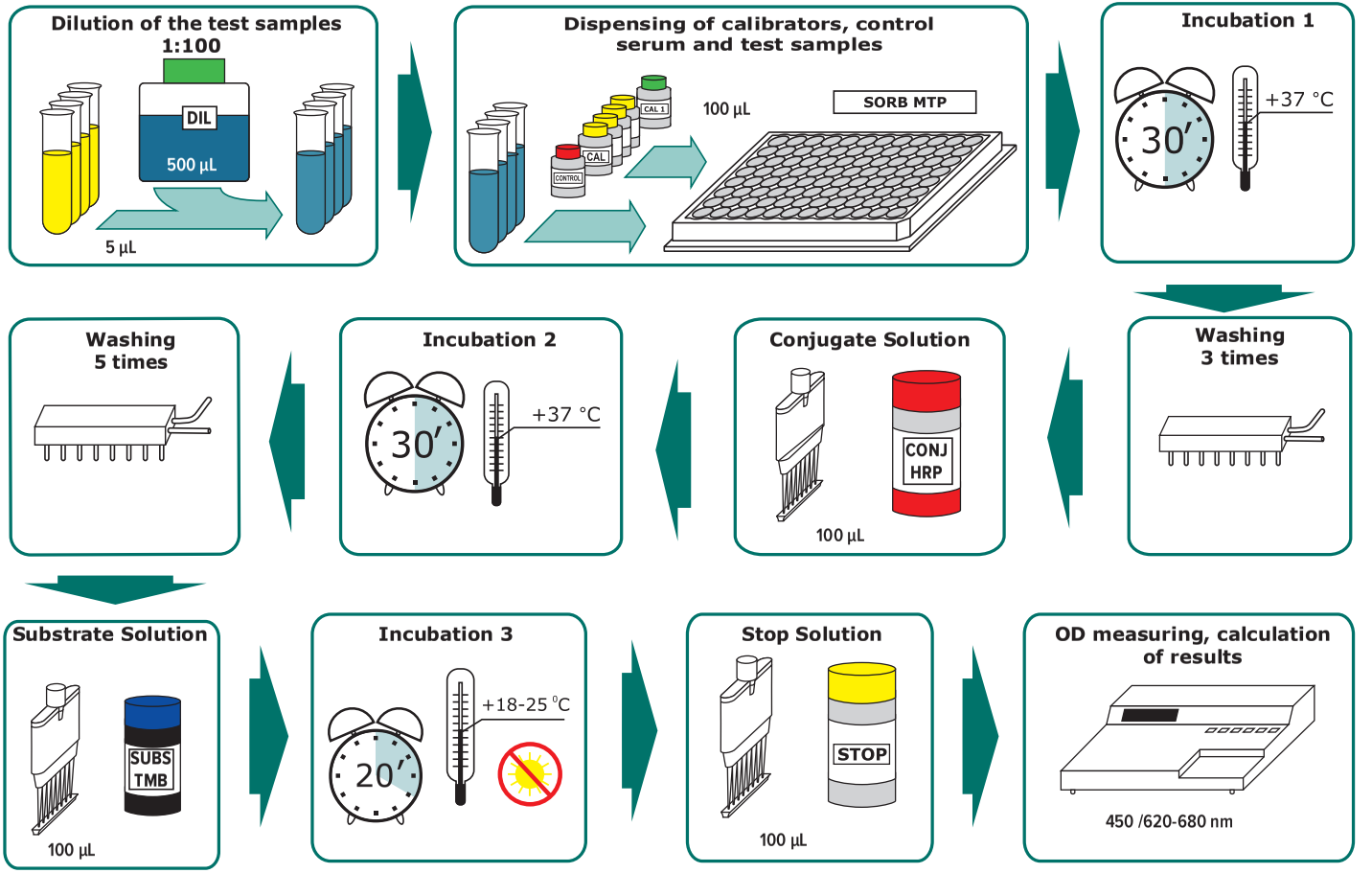
UA.TR.099

ASSAY PROCEDURE



K103

CONTENT

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

1. INTENDED USE

The Cytomegalovirus IgG EIA kit is an enzyme immunoassay, intended for the quantitative and/or qualitative determination of IgG antibodies concentration to cytomegalovirus in human serum or plasma.

The Cytomegalovirus IgG EIA kit is used for the diagnosis of cytomegalovirus infection.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Human cytomegalovirus (CMV) belongs to the herpesvirus family, a member of the genus Herpesviridae known as human herpesvirus 5 (HHV-5). It is a widespread virus, the manifestations of which are characterized by a mild, asymptomatic course in people with a healthy immune system. The infection is of particular clinical importance in people with immune system disorders, immunodeficiency states and pregnant women due to the risk of intrauterine infection of the fetus.

The source of infection is a person who excretes the virus through various biological fluids. The infection is transmitted through airborne droplets, hemocontact, sexual contact, and blood transfusion from an infected donor. Infection is also possible during organ transplantation and through transmission from an infected woman to a child during pregnancy, childbirth, or breastfeeding.

The duration of the incubation period of the disease is often impossible to establish, as most clinical cases go unrecognized and have minor clinical symptoms, after which they become latent. If the immune system is weakened, the disease can be severe. The main symptoms may include fatigue, fever, fever and/or sweating, muscle pain, decreased appetite, swollen lymph nodes, headache, and an acute respiratory disease clinic in the form of pharyngitis, laryngitis, bronchitis, etc.

Transplacental infection of the fetus is possible both in the case of primary infection in the mother and as a result of reactivation of chronic infection. The virus can affect any fetal organ, including the central nervous system. Newborns with an acute form of infection may have rashes on the arms and legs, face, and trunk. There may also be hemorrhages, blood in the stool, sometimes seizures and drowsiness.

The main goal of CMV treatment is to eliminate the symptoms of the disease. A very important condition for the successful treatment of the disease is to increase immunity. Drug therapy can increase the period of remission and affect the recurrence of infection, but does not eliminate the virus from the body.

Laboratory diagnosis of infection is based on various methods of detecting CMV, its antigens and specific antibodies. These include: molecular hybridization, PCR, which allow detecting viral DNA directly in the samples under study; cytoscopy of saliva, urine, breast milk, cerebrospinal fluid sediments, which allows detecting transformed cells with a large nucleus and a narrow cytoplasmic border; enzyme-linked immunosorbent assay (ELISA), which is the most sensitive method for detecting IgM and IgG antibodies, etc.

Determination of antibodies by ELISA allows to detect latent, primary CMV infection or reactivation of the virus in the presence of seroconversion, to determine the titer of antibodies in the dynamics, to establish a risk group among pregnant women and women planning pregnancy.

In the first days after the onset of the infection, the production of IgM antibodies begins, then their number increases during the first two weeks, then gradually decreasing after a few months. The presence of these antibodies indicates a fresh infection or reactivation of latent and persistent forms of infection. IgG antibodies to CMV appear 2-4 weeks after infection, their level in the blood gradually increases and can persist for a long period of time.

3. PRINCIPLE OF THE TEST

The determination of of IgG antibodies to cytomegalovirus is based on the indirect enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized antigen cytomegalovirus. 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 cytomegalovirus IgG 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 (OD) in the microwell is directly related to the quantity of the measured IgG antibodies to cytomegalovirus in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density of IgG antibodies to the CMV antigen in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P103Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with antigen cytomegalovirus, ready to use
C103Z	CAL 1	**Calibrator C1**	1.5 mL	1	Solution based on tris buffer (pH 7.2-7.4), free of IgG antibodies to CMV, with preservative, ready to use (colourless liquid)
C103Z	CAL 2-5	**Calibrators**	1.5 mL	4	Solutions based on tris buffer (pH 7.2-7.4), containing 0,5; 1,5; 3 and 6 U/mL of IgG antibodies to CMV, with preservative, ready to use (blue liquids)
Q103Z	CONTROL	**Control Serum**	1.5 mL	1	Solution based on human serum, containing of known IgG antibodies to CMV content, with preservative, ready to use (colourless liquid)
T103Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of murine monocnoclonal antibodies to IgG conjugated to the horseradish peroxidase; ready to use (red 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 Cytomegalovirus IgG EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

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

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

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

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

- 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).

Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

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								

For qualitative results: add **100 µL of Calibrators** CAL1, CAL2, and CAL5 in duplicate to the appropriate wells. To the remaining wells, add **100 µL of diluted serum (plasma) samples** in duplicate. The addition of Calibrators and test samples should be performed within 5 minutes.

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

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

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

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

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

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

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. For quantitative results: plot a calibration curve in linear coordinates: (x) is the concentration of IgG antibodies to CMV U/mL in the calibrators, (y) – OD of Calibrators (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.
Determine the corresponding concentration of IgG antibodies to CMV in tested samples using the calibration curve.

10.13. For qualitative results:
- calculate the mean OD value of the Calibrator CAL2;
- multiply the Q coefficient given in the quality control datasheet to the mean CAL2 value, obtaining the optical density limit value (ODL);
- for each sample, calculate the K-factor by dividing the sample OD value by the ODL.

samples with K > 1.1 should be considered **POSITIVE**,
samples with K from 0.91 to 1.09 should be considered **EQUIVOCAL**,
samples with K < 0.9 should be considered **NEGATIVE**.

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 CMV IgG. Based on data obtained by XEMA, the following normal range is recommended (see below).

12.2. Some laboratories, based on their population studies, set up a «second cut-off», which stands between anamnestic («normal») IgG antibody level and «high» IgG antibody level characteristic of reactivation or late period of primary infection. Recommended values for this second cut-off for two age groups (8 months – 3 year, > 3 years) are presented in the table below.

If the value is between 0.6 U/mL (K from 1.1) to the «second cut-off», this may indicate either the initial period of primary infection or a previously transferred infection. It is recommended to test samples from the same patient collected a few weeks later. An increasing titer in the repeated sample indicates the presence of infection. If the titer does not increase, this indicates the absence of an active infection and the anamnestic nature of the antibodies.

If the concentration in the test sample exceeds the value of the upper calibration sample (6.0 U/mL), it should be additionally diluted by 10 times or more with EIA buffer. To calculate the concentration, multiply the result by the dilution factor.

Sex, age	Units, U/mL		Units alternative, K	
	Lower limit	Upper limit	Lower limit	Upper limit
seronegative	< 0.1	0.5	< 0.1	0.9
seropositive > 3 years	0.6	2.7	1.1	4.9
newborn*	< 0.1	0.9	< 0.1	1.7
under 8 months*	< 0.1	1.9	< 0.1	3.5
8 months – 3 years	< 0.1	3.1	< 0.1	5.5

* antibodies of maternal origin.

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1. Precision of Measurement

Reproducibility. The coefficient of variation of detection of IgG antibodies to CMV in the same sample of human serum or plasma using the kit Cytomegalovirus IgG EIA does not exceed 10%.

13.1.2. 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.

13.1.3. Linearity

The dependence of the concentration of IgG antibodies to CMV antigens in blood serum (plasma) samples diluted with blood serum (plasma) that is free of IgG antibodies to CMV antigens is linear in the concentration range of 0.5-6 U/mL and is ±10%.

14. LIMITATIONS

A positive result in the Cytomegalovirus IgG EIA kit indicates the presence of specific IgG antibodies to CMV. In some cases, in the early stages of the disease, the EIA result may be negative due to the absence or ultra-low titer of antibodies below the limit of detection of the assay. In such cases, if symptoms of the disease are present, it is recommended to re-sample and test the sample in 7-10 days, additionally to analyze the sample for the content of IgM antibodies (for example, using the kit Cytomegalovirus IgG EIA, manufactured by XEMA LLC), and to verify the result by another laboratory method (for example, by PCR using the kit Cytomegalovirus PCR, manufactured by XEMA LLC).

The final diagnosis cannot be established on the basis of the detection IgG antibodies to CMV results and requires confirmation, in particular, an assessment of the clinical manifestations of the disease, the patient's history, and laboratory test results.

15. REFERENCES

1. Gupta M, Shorman M. Cytomegalovirus. 2021 Aug 11. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 29083720.
2. Novak, Z., Ross, S. A., Patro, R. K., Pati, S. K., Reddy, M. K., Purser, M., Britt, W. J., & Boppana, S. B. (2009). Enzyme-linked immunosorbent assay method for detection of cytomegalovirus strain-specific antibody responses. Clinical and vaccine immunology: CVI, 16(2), 288–290. https://doi.org/10.1128/CVI.00281-08.
3. Revello M.G., Gema G. Diagnosis and management of human cytomegalovirus infection in the mother, fetus and newborn infant // Clin. Microbiol. Rev. – 2002-v.15, no.4 – p.680-715.
4. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
5. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
6. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

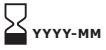
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

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

LOT _____ DATE _____

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

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

+38 044 294-69-78

or write to:

qa@xema.com.ua

XEMA LLC
Akademika Yefremova St. 23
03179, Kyiv, Ukraine
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tel.:+38 044 294-69-78
E-mail: qa@xema.com.ua
www.xema.com.ua

Instruction for use
A solid-phase enzyme immunoassay kit
for the qualitative detection of
IgM antibodies to cytomegalovirus
in human serum or plasma

Cytomegalovirus IgM EIA

Catalogue number REF **K103M**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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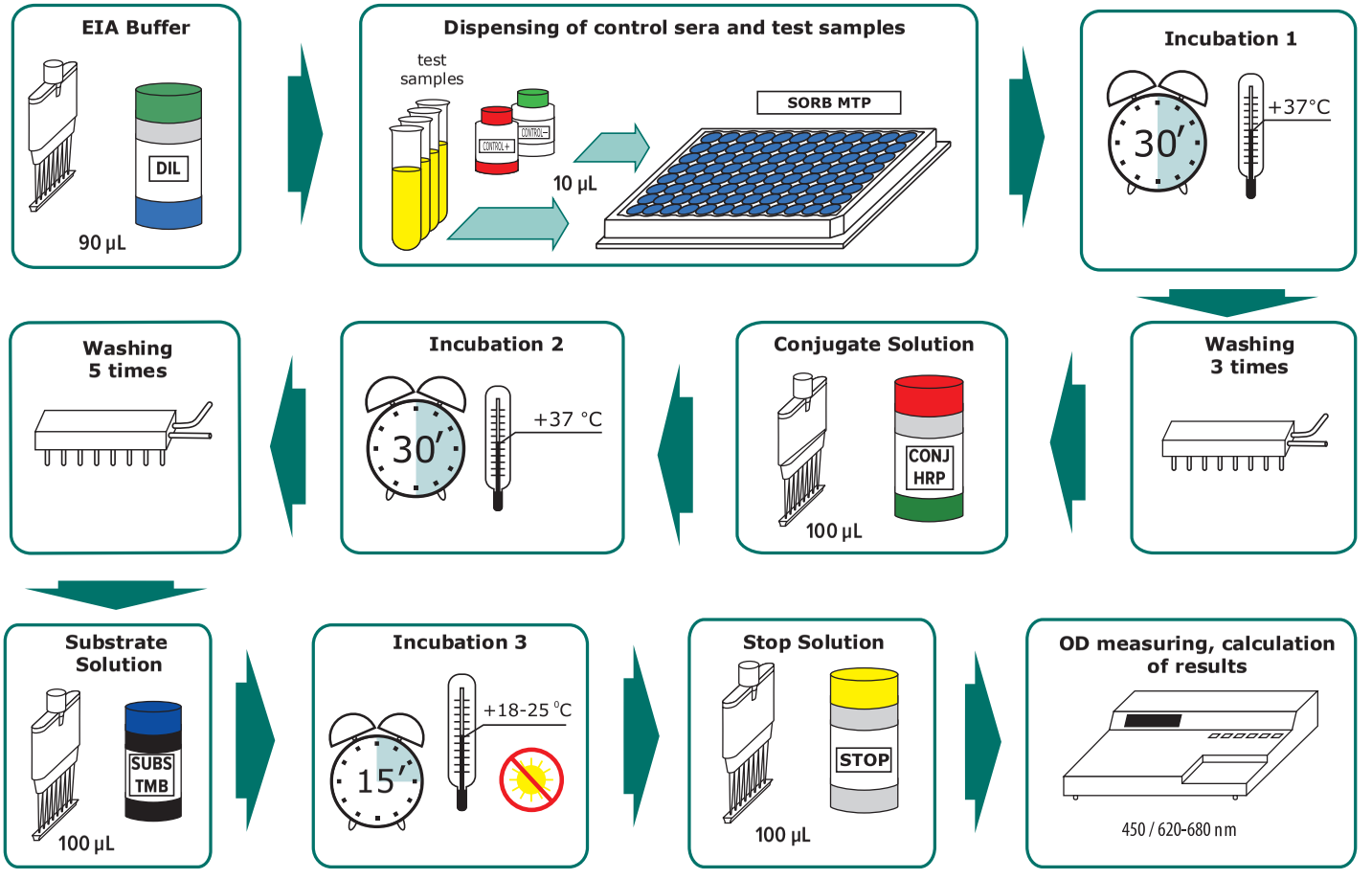
UA.TR.099

ASSAY PROCEDURE



K103M

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the qualitative detection of IgM antibodies to cytomegalovirus in human serum or plasma
Cytomegalovirus IgM EIA

1. INTENDED USE

The Cytomegalovirus IgM EIA kit is an enzyme immunoassay, intended for the qualitative detection of IgM antibodies to cytomegalovirus in human serum or plasma.

The Cytomegalovirus IgM EIA kit is used for the diagnosis of cytomegalovirus infection.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Human cytomegalovirus (CMV) belongs to the herpesvirus family, a member of the genus Herpesviridae known as human herpesvirus 5 (HHV-5). It is a widespread virus, the manifestations of which are characterized by a mild, asymptomatic course in people with a healthy immune system. The infection is of particular clinical importance in people with immune system disorders, immunodeficiency states and pregnant women due to the risk of intrauterine infection of the fetus.

The source of infection is a person who excretes the virus through various biological fluids. The infection is transmitted through airborne droplets, hemocontact, sexual contact, and blood transfusion from an infected donor. Infection is also possible during organ transplantation and through transmission from an infected woman to a child during pregnancy, childbirth, or breastfeeding.

The duration of the incubation period of the disease is often impossible to establish, as most clinical cases go unrecognized and have minor clinical symptoms, after which they become latent. If the immune system is weakened, the disease can be severe. The main symptoms may include fatigue, fever, fever and/or sweating, muscle pain, decreased appetite, swollen lymph nodes, headache, and an acute respiratory disease clinic in the form of pharyngitis, laryngitis, bronchitis, etc.

Transplacental infection of the fetus is possible both in the case of primary infection in the mother and as a result of reactivation of chronic infection. The virus can affect any fetal organ, including the central nervous system. Newborns with an acute form of infection may have rashes on the arms and legs, face, and trunk. There may also be hemorrhages, blood in the stool, sometimes seizures and drowsiness.

The main goal of CMV treatment is to eliminate the symptoms of the disease. A very important condition for the successful treatment of the disease is to increase immunity. Drug therapy can increase the period of remission and affect the recurrence of infection, but does not eliminate the virus from the body.

Laboratory diagnosis of infection is based on various methods of detecting CMV, its antigens and specific antibodies. These include: molecular hybridization, PCR, which allow detecting viral DNA directly in the samples under study; cytoscopy of saliva, urine, breast milk, cerebrospinal fluid sediments, which allows detecting transformed cells with a large nucleus and a narrow cytoplasmic border; enzyme-linked immunosorbent assay (ELISA), which is the most sensitive method for detecting IgM and IgG antibodies, etc.

Determination of antibodies by ELISA allows to detect latent, primary CMV infection or reactivation of the virus in the presence of seroconversion, to determine the titer of antibodies in the dynamics, to establish a risk group among pregnant women and women planning pregnancy.

In the first days after the onset of the infection, the production of IgM antibodies begins, then their number increases during the first two weeks, then gradually decreasing after a few months. The presence of these antibodies indicates a fresh infection or reactivation of latent and persistent forms of infection. IgG antibodies to CMV appear 2-4 weeks after infection, their level in the blood gradually increases and can persist for a long period of time.

3. PRINCIPLE OF THE TEST

The detection of IgM antibodies to cytomegalovirus is based on IgM-capture enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized anti-species murine monoclonal antibodies to IgM. CMV antigens conjugated to the horseradish peroxidase are used as enzyme conjugate. The analysis procedure includes three stages of incubation:

- during the first stage IgM antibodies from the specimen are bound by anti-species murine monoclonal antibodies coated onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated CMV antigen bind to bind to specific IgM antibodies, 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 (OD) in the microwell is directly related to the quantity of the measured IgM antibodies to cytomegalovirus in test specimen.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P103MZ	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to IgM, ready to use
CN103MZ	CONTROL -	**Negative Control Serum K-**	0.5 mL	1	Solution based on human serum, free of IgM antibodies to CMV, with preservative, ready to use (yellow liquid)
CP103MZ	CONTROL +	**Positive Control Serum K+**	0.2 mL	1	Solution of IgM antibodies to CMV antigens, with preservative, ready to use (red liquid)
T103MZ	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of CMV antigens conjugated to the horseradish peroxidase, ready to use (green liquid)
S011Z	DIL	**EIA Buffer**	14 mL	1	Buffer solution with detergent and preservative, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution, ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	2	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid? ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (3 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm 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 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 Cytomegalovirus IgM 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 Cytomegalovirus IgM 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 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 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 and 4 wells for Positive and Negative Control Serum (1 well for Positive Control (CP) and 3 wells for Negative Control Serum (CN)).

10.2. Dispense **90 µL of EIA Buffer** to all wells.

10.3. Dispense **10 µL of Positive and Negative Control Serum as well as 10 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Positive and Negative 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, Positive and Negative 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	CP	SAMP5	SAMP13	SAMP21								
B	CN	SAMP6	SAMP14	SAMP22								
C	CN	SAMP7	SAMP15	SAMP23								
D	CN	SAMP8	SAMP16									
E	SAMP1	SAMP9	SAMP17									
F	SAMP2	SAMP10	SAMP18									
G	SAMP3	SAMP11	SAMP19									
H	SAMP4	SAMP12	SAMP20									

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 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 in the wells 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**. *The incubation time can be changed depending on the level of blue color development.*

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.

11. TEST VALIDITY AND CALCULATION OF RESULTS

11.1. The test results are valid only if Positive and Negative Control Serum are within the specified ranges and if all other test parameters are also within the given assay specifications, namely:

- OD of CONTROL- < 0.15;
- OD of CONTROL+ > 1.0.

11.2. Calculate the mean OD value of the Negative Control Serum:

meanOD(CN) = (OD1(CN)+OD2(CN) + OD3(CN))/3

If one of the OD values of the Negative Control Serum differs significantly, it should be discarded and the meanOD(CN) should be calculated using the remaining OD values of the Negative Control Serum.

11.3. Calculate the Cut-Off value by adding to the mean OD value of the Negative Control Serum the coeficient 0.3.

Cut-off = meanOD(CN) + 0.3

11.4. Calculate Positivity Index (PI) for each sample by dividing the OD of the sample by Cut-off value:

PI = ODsample/Cut off

12. INTERPRETATION OF THE RESULTS

If PI value > 1.1 the result is **POSITIVE**,
If PI value is between 0.9 and 1.1 the result is **EQUIVOCAL**,
If PI value < 0.9 the result is **NEGATIVE**.

If equivocal results are obtained, it is recommended to conduct a reexamination of the sample in several replicates. If the result is equivocal again, a new sample should be obtained within 5-7 days and retested. If the result remains equivocal, the sample should be considered negative.

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

Precision of Measurement

Reproducibility. The coefficient of variation of detection of IgM antibodies to CMV in the same sample of human serum or plasma using the kit Helicobacter pylori IgM EIA does not exceed 8%.

Inter assay reproducibility was determined by evaluating the coefficients of variation (CV) for 2 samples during 3 days in 8-replicate determinations

No. sample	mean PI	CV1, %
1	0.54	8.7
2	1.98	6.1

Repeatability (Intra assay repeatability) was determined by evaluating the coefficients of variation (CV) for 2 samples during 1 day in 32 replicates on two series of ELISA kit.

No. sample	mean PI	CV1, %	CV2, %
1	0.504	5.9	6.5
2	1.908	3.7	3.5

13.2. Diagnostic performance characteristics

Sensitivity and specificity. Sensitivity and specificity of the kit Cytomegalovirus IgM EIA was evaluated using a serum panel Anti-CMV Mixed Titer Performance Panel PTC202(M) of Boston Biomedica Company (США) production вin accordance with the passport data and correlates with the values obtained on the kit Abbott EIA CMV-IgM (lot 44423M100). The panel contains 3 positive and 8 negative samples. The clinical sensitivity and specificity on the commercial panel was 100%.

In addition, to determine the relative specificity of the kit, 52 human sera were tested against commercial kits. All samples were determined as negative.

14. LIMITATIONS

A positive result of the test indicates that the patient has IgM antibodies specific to CMV antigens. In some cases, in the early stages of the disease, the ELISA result may be negative due to the absence or ultra-low titer of antibodies below the limit of sensitivity of the test. In such cases, if symptoms of the disease are present, it is recommended to re-sample and analyze the sample in 7-10 days, additionally to test the sample for the content of IgG antibodies (for example with Cytomegalovirus IgG EIA, manufactured by XEMA LLC), and also to verify the result by another laboratory method (for example, by PCR with Cytomegalovirus PCR, manufactured by XEMA LLC).

Both laboratory test results and clinical manifestations of the disease should be taken into account for the establishment of the diagnosis.

15. REFERENCES

1. Gupta M, Shorman M. Cytomegalovirus. 2021 Aug 11. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan–. PMID: 29083720.
2. Novak, Z., Ross, S. A., Patro, R. K., Pati, S. K., Reddy, M. K., Purser, M., Britt, W. J., & Boppana, S. B. (2009). Enzyme-linked immunosorbent assay method for detection of cytomegalovirus strain-specific antibody responses. Clinical and vaccine immunology: CVI, 16(2), 288–290. https://doi.org/10.1128/CVI.00281-08.
3. Revello M.G., Gema G. Diagnosis and management of human cytomegalovirus infection in the mother, fetus and newborn infant // Clin. Microbiol. Rev. – 2002- v.15, no.4 – p.680-715.
4. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
5. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
6. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

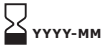
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

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

LOT _____ DATE _____

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

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

+38 044 294-69-78

or write to:

qa@xema.com.ua

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www.xema.com.ua

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

Testosterone EIA

Catalogue number REF **K209**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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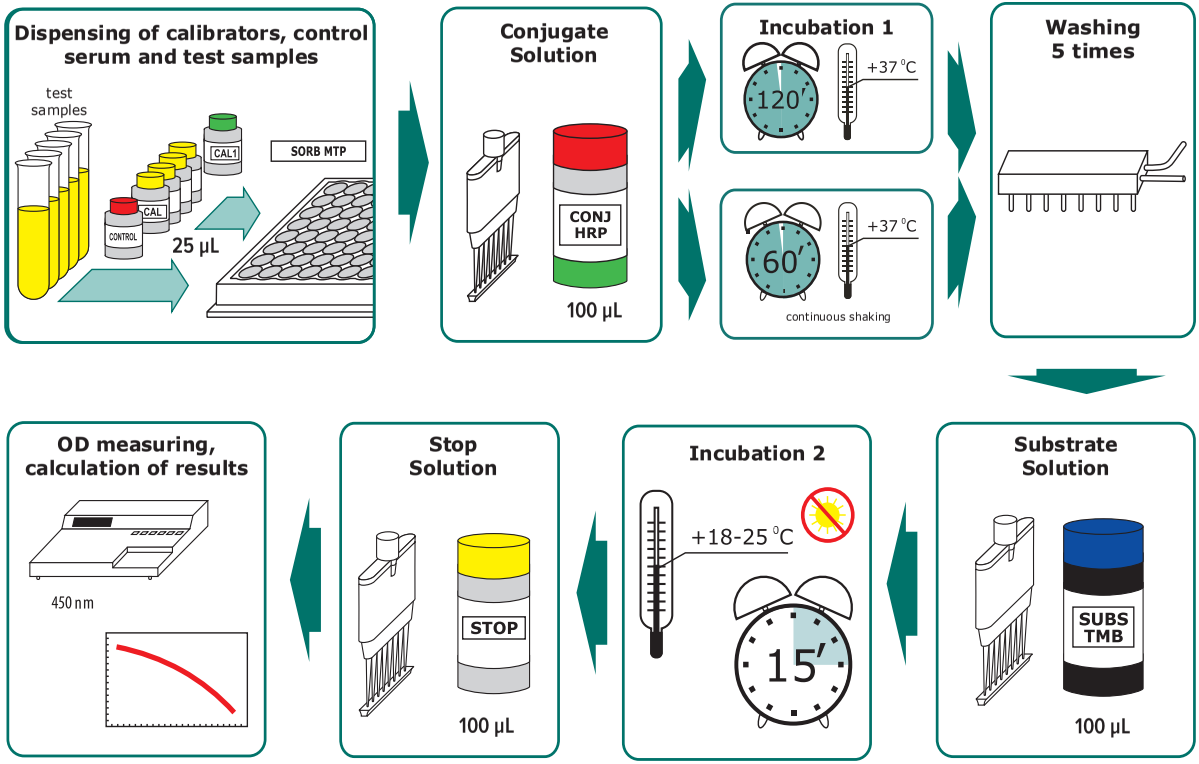
ASSAY PROCEDURE



K209

CONTENT

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

1. INTENDED USE

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

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

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

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

Leidig cell tumours producing high levels of serum testosterone in young boys lead to development of "little Hercules" syndrome. Elevated testosterone level in women causes the clinical signs of masculinization.

In men, decreased testosterone levels may lead to female habitus or underdevelopment of male genital organs in boys. To differentiate between primary and secondary hypogonadism, testosterone should be assayed in conjunction with LH and FSH.

3. TEST PRINCIPLE

The determination of the testosterone is based on the competition principle of the enzyme immunoassay. On the inner surface of the microplate wells are immobilized specific to testosterone murine monoclonal antibodies. Testosterone conjugated to the horseradish peroxidase is used as enzyme conjugate.

The analysis procedure includes two stages of incubation:

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

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

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P209Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to testosterone; ready to use
C209Z	CAL 1	**Calibrator C1**	0.5 mL	1	Solution based on human plasma, free of testosterone, with preservative, ready to use (colourless liquid)
C209Z	CAL 2-6	**Calibrators**	0.5 mL	5	Solutions based on human plasma, containing 1; 3; 10; 30 and 100 nmol/L of testosterone, with preservative, ready to use (blue liquids)
Q209Z	CONTROL	**Control serum**	0.5 mL	1	Solution based on human serum, containing of known testosterone content, with preservative, ready to use (blue liquid)
T209Z	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of testosterone conjugated to the horseradish peroxidase; ready to use (green liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (1 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm wavelength;
- dry thermostat for +37 °C±2°C or thermostat shaker maintaining a speed of 600 rpm and temperature of +37°C ±1°C;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- vortex mixer;
- disposable gloves;
- absorbent paper.

6. WARNING AND PRECAUTIONS

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

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

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

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

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

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

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

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

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

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

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

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

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

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

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

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

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

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

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

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

8.1. Transportation

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

8.2. Storage

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

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

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

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

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

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

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

8.3. Disposal

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

9. REAGENTS PREPARATION

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

9.2. Microplate preparation

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

9.3. Washing Solution preparation

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

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

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

10. ASSAY PROCEDURE

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

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

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

Scheme of introduction of samples

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

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

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **120 minutes at +37°C**. Incubation for 60 minutes at +37°C with continuous shaking 600 rpm is allowed.

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

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

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

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

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

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

11. TEST VALIDITY

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

12. EXPECTED VALUES

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

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

12.2. The calibrators concentration values of the Testosterone EIA kit are expressed in nmol/L. To calculate concentrations in ng/mL, the received concentration value in nmol/L shall be multiplied by 0.29.

1 nmol/L = 0,29 ng/mL

Sex, age	Units, nmol/L		Units alternative, ng/mL	
	Lower limit	Upper limit	Lower limit	Upper limit
Males				
20-39 yrs	9.0	38	2.6	11
40-55 yrs	6.9	21	2.0	6.1
>55 yrs	5.9	18.1	1.7	5.2
Females	-	4.6	-	1.3

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

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

Sample	Concentration, nmol/L	CV, %
1	93.16	1.63
2	28.5	7.87

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

Sample	Concentration, nmol/L	CV, %
1	95.34	5.25
2	28.47	2.57

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

Sample	Concentration1, nmol/L	Concentration2, nmol/L	Concentration3, nmol/L	CV, %
1	94.6	95.89	97.89	1.72
2	28.4	27.75	29.46	3.02

13.1.2 Trueness

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

13.1.3 Linearity

Linearity was determined using sera samples with known testosterone concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 1–100nmol/L ±10%.

13.1.4 Analytical sensitivity

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

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for Testosterone EIA kit is 1.0 nmol/L.

13.1.5 Analytical specificity

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

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

Analyte	Cross-reactivity, %
5-alpha-dehydrotestosterone	16
Androstendiol	1
Androstendione	0.4
Androsterone	<0.1
Dehydroepiandrosterone	<0.1
Progesterone	<0.1
Estradiol, Estriol	<0.01
Cortisol, Pregnenolone	<0.01

14. REFERENCES

1. Tietz, N.W. Textbook of Clinical Chemistry. Saunders, 1986.
2. Joshi, U. M., et al. Steroids 34 (1) 35 (1979).
3. Turkes, A., et al. J Endocrinol. 81 (2) P165 (1979).
4. Ismail, A. A., Niswender, G. D. Midgley, A. R. J. Clin. Endocr. Metab. 34, 177 – 184 (1972).
5. Rajkowski,K. M.,Cittanova N.,Desfosses, B.and Jayle, M.F. Steroids 29 no 5 1977 6. Widsdom G. B. Clin. Chem. 22/8, 1243 – 1255 (1976).
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

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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:

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

DHEAS EIA

Catalogue number REF **K215**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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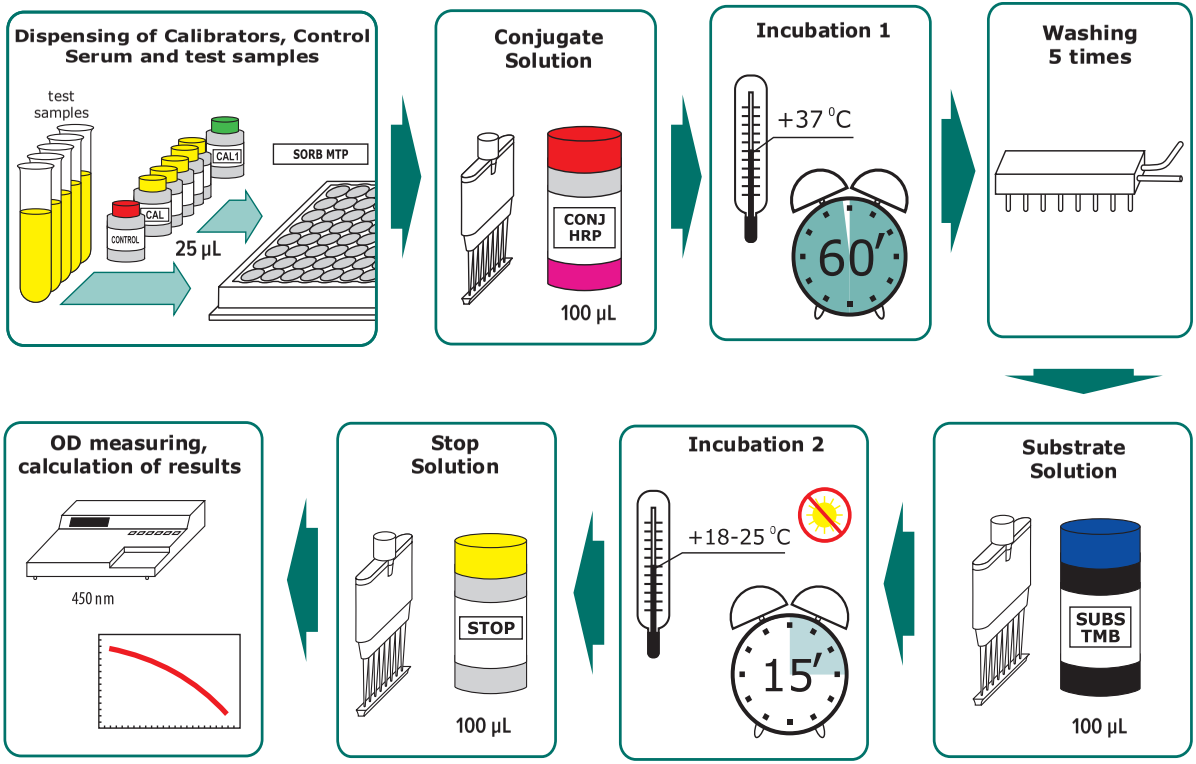
tel.:+ 49 911 931 639 67
E-mail: info@polmed.de
www.polmed.de

ASSAY PROCEDURE



K215

CONTENT

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

1. INTENDED USE

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

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Dehydroepiandrosterone (DHEA) is an androgen with a MW of 288.4 Dalton secreted in adrenals. The main derivate of DHEA present in human tissue is DHEA sulfate (DHEAS). Since birth, DHEAS serum concentration is increasing continuously showing a pronounced peak after puberty and maximal levels at the age of 20. After that, serum DHEAS level continuously decreases. As DHEAS is the main component of 17-ketosteroids in serum, this test may substitute for column tests for determination of 17-ketosteroids in urine.

Elevated DHEAS concentrations are found in adrenogenital syndrome, hirsutism, acne, benign hyperplasia of adrenals and adrenal tumors, Stein-Leventhal syndrome, polycystic ovary syndrome.

Decreased levels of DHEAS are found in hyperlipidemia, psychotic states, psoriasis, adrenal insufficiency.

3. TEST PRINCIPLE

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

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

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

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

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P215Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with rabbit polyclonal antibodies to DHEAS; ready to use
C215Z	CAL 1	**Calibrator C1**	0.5 mL	1	Solution based on human plasma, free of DHEAS, with preservative, ready to use (yellow liquid)
C215Z	CAL 2-6	**Calibrators**	0.5 mL	5	Solutions based on human plasma, containing 0,1; 0.3; 1; 3 and 10 µg/mL of DHEAS, with preservative, ready to use (blue liquids)
Q215Z	CONTROL	**Control Serum**	0.5 mL	1	Solution based on human plasma, containing of known DHEAS content, with preservative, ready to use (colourless liquid)
T215Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of DHEAS conjugated to the horseradish peroxidase; ready to use (magenta liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

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

6. WARNING AND PRECAUTIONS

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

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

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

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

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

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

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

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

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

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

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

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

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

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

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

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

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

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

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

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

8.1. Transportation

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

8.2. Storage

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

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

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

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

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

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

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

8.3. Disposal

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

9. REAGENTS PREPARATION

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

9.2. Microplate preparation

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

9.3. Washing Solution preparation

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

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

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

10. ASSAY PROCEDURE

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

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

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

Scheme of introduction of samples

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

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

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

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

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

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

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

10.9 Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the DHEAS concentration in the calibrators µg/mL, (y) – OD versus DHEAS concentration (OD 450 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve. Adjust the concentration of CAL1 to an infinitesimally small value, for example, 0.001 µg/mL.

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

11. TEST VALIDITY

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

12. EXPECTED VALUES

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

NOTE: values of DHEAS concentrations in the tested samples that are below the LoD (0.025 µg/mL) and also exceed the value of the upper calibrator (10 µg/mL) should be provided in the following form : «the DHEAS concentration of tested sample X is «lower than 0.025 µg/mL» or «higher than 10 µg/mL».

12.2 The calibrators concentration values of the DHEAS EIA kit are expressed in µg/mL. To calculate concentrations in µmol/L, the received concentration value in µg/mL shall be multiplied by 2.6.

1 µg/mL = 2.6 µmol/L

Sex, age	Units, µg/mL		Units alternative, µmol/L	
	Lower limit	Upper limit	Lower limit	Upper limit
Males				
newborn	1.08	4.06	2.81	10.56
1 month-5 yrs	0.01	0.41	0.03	1.07
6-9 yrs	0.03	1.45	0.07	3.77
10-11 yrs	0.12	1.15	0.31	2.99
12-17 yrs	0.2	5.55	0.52	14.43
18-30 yrs	1.25	6.19	3.25	16.09
31-50 yrs	0.59	4.52	1.53	11.75
51-60 yrs	0.2	4.13	0.52	10.74
>61 yrs	0.1	2.35	0.26	6.11
Females				
newborn	0.1	2.48	0.26	6.45
1 month-5 yrs	0.05	0.55	0.13	1.43
6-9 yrs	0.03	1.4	0.07	3.64
10-11 yrs	0.15	2.6	0.39	6.76
12-17 yrs	0.2	5.35	0.52	13.91
18-30 yrs	0.29	7.81	0.75	20.31
31-60 yrs	0.12	3.79	0.31	9.85
post menopausal	0.3	2.6	0.78	6.76
Pregnancy week				
1st trimester	0.38	3.6	0.99	9.36
2nd trimester	0.42	3.0	1.09	7.8
3rd trimester	0.32	2.5	0.83	6.5

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

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

Sample	Concentration, µg/mL	CV, %
1	4.02	5.9
2	3.38	7.34

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

Sample	Concentration, µg/mL	CV, %
1	2.49	6.12
2	4.23	7.41

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

Sample	Concentration, µg/mL	Concentration, µg/mL	Concentration, µg/mL	CV, %
1	1.98	1.89	2.03	11.45
2	1.69	1.78	1.64	13.6

13.1.2 Trueness

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

13.1.3 Linearity

Linearity was determined using sera samples with known DHEAS concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 0.1-10 µg/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest DHEAS concentration in the serum or plasma sample that is detected by the DHEAS EIA kit is no lower than 0.05 µg/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for DHEAS EIA kit is 0.08 µg/mL.

13.1.5 Analytical specificity

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

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

Analyte	Cross-reactivity, %
DHEA	50
other steroids	<0,01

14. REFERENCES

1. P. Tijssen, «Practice & Theory of Enzyme Immunoassays», (1985) Amsterdam: Elsevier.
2. E. Friess, et al., Eur J Clin Invest, (2000) 30 Suppl 3:46-50.
3. C. Longscope, J Endocrinology, (1996) 150 Suppl: S125-S127.
4. J. Herbert, Lancet, (1995) 345:1193-1194.
5. A. Michael, et al., Biol. Psychiatry, (2000) 48(10): 989-95.
6. C.R. Dequet and D.J. Wallace, Current Opin Invest Drugs, (2001) 8: 1045-53.
7. W.M. Jeffries., Med Hypotheses, (1998) 51(2):111-4.
8. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.
9. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
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LOT _____ DATE _____

	Manufacturer
	*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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Instruction for use
A solid-phase enzyme immunoassay kit
for the quantitative determination of
17-OH-progesterone in human serum or plasma

17-OH-progesterone EIA

Catalogue number REF **K217**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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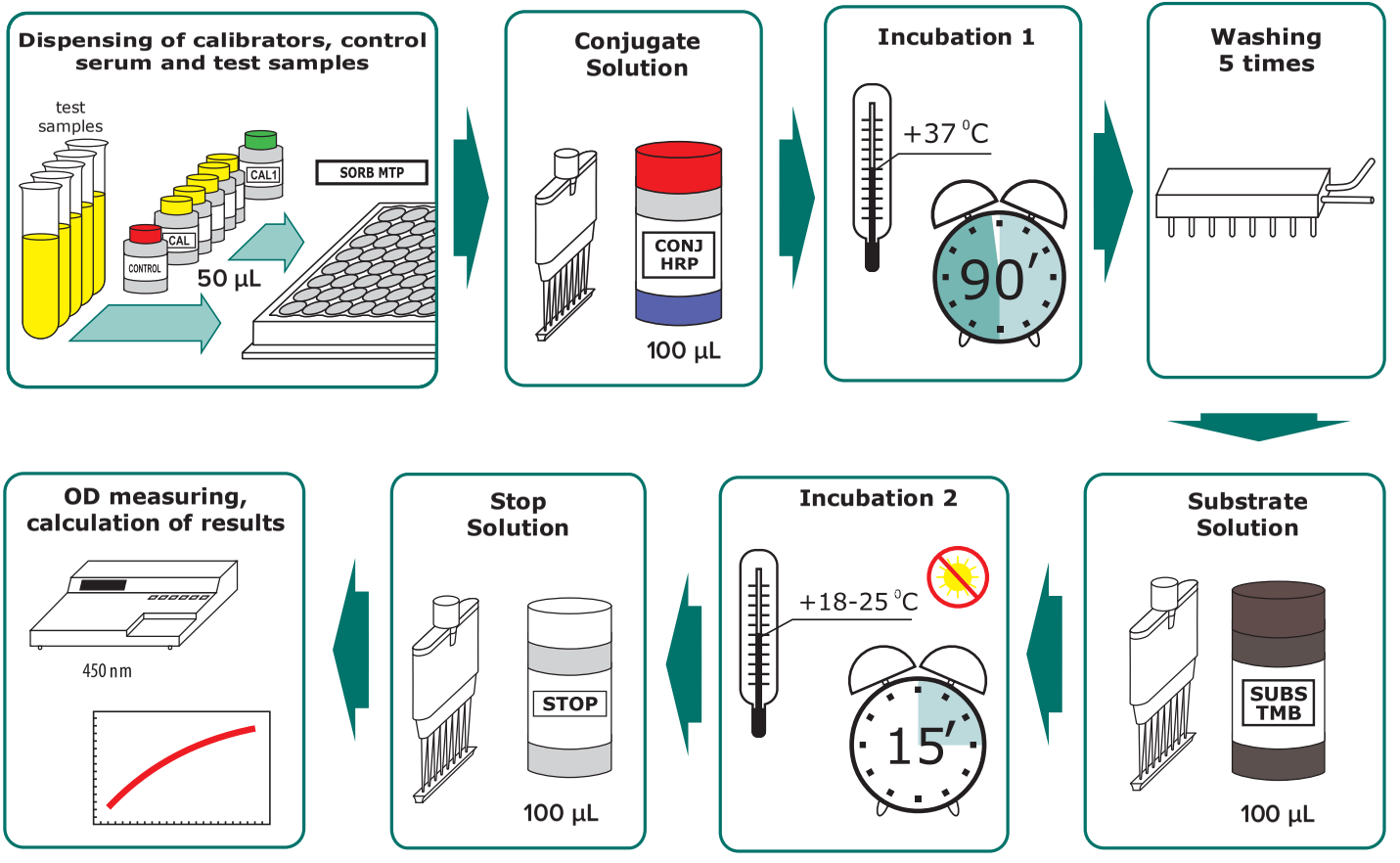
ASSAY PROCEDURE



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

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of 17-OH-progesterone in human serum or plasma

17-OH-progesterone EIA

1. INTENDED USE

The 17-OH-progesterone EIA kit is an enzyme immunoassay, intended for the quantitative determination of 17-OH-progesterone in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

17-OH-progesterone (17-OH-P) is a steroid with molecular weight 330 Da, an intermediate precursor in biosynthesis of glucocortiosteroids, estrogens and androgens.

17-OH-P is secreted by adrenals, ovaries and testicles by the enzyme 21-hydroxylase. 17-OH-P is secreted by ovaries during follicular phase; its serum level remains stable by the end of luteal phase. In case of non-fertile cycle, the serum level of 17-OH-P decreases; in case of fertilization, this hormone is secreted by corpus luteum.

The determination of 17-OH-P is important for diagnosis of inborn adrenal hyperplasia which causes elevated secretion of androgens and the development of adrenogenital syndrome (AGS). In AGS, the deficient 21-hydroxylase activity causes blocked steroid synthetic pathway and correspondent dramatic increase in serum 17-OH-P level. If the deficiency of 21-hydroxylase is acquired in mature age, or in case of delayed inborn defect, the serum 17-OH-P may remain normal.

3. TEST PRINCIPLE

Determination of the 17-OH-progesterone is based on competition principle of the enzyme immunoassay. Microwells plate is coated with the polyclonal rabbit antibodies to the 17-OH-progesterone. 17-OH-progesterone conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage 17-OH-progesterone from the specimen competes with the conjugated 17-OH-progesterone for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed;
- during the second stage, the complexes formed due 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 17-OH-progesterone 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 17-OH-progesterone in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P217Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with polyclonal rabbit antibodies to the 17-OH-progesteron, ready to use
C217Z	CAL 1	**Calibrator C1**	0.6 mL	1	Solution based on human plasma, free of 17-OH-progesteron, with preservative, ready to use (colourless or yellow liquid)*
C217Z	CAL 2-6	**Calibrators**	0.6 mL	5	Solutions based on human plasma, containing 0.5; 1.5; 5; 20 and 100 nmol/L of 17-OH-progesteron, with preservative, ready to use (magenta liquids)*
Q217Z	CONTROL	**Control Serum**	0.6 mL	1	Solution based on human plasma, containing of known 17-OH-progesteron content, with preservative, ready to use (ccolourless or yellow liquid)*
T217Z	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of 17-OH-progesterone conjugated to the horseradish peroxidase, ready to use (magenta 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). *- slight sedimentation is allowed, which does not affect the performance of calibration samples and control serum.					

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

8.2. Storage

The 17-OH-progesterone EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

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

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

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

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

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

8.3. Disposal

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

9. REAGENTS PREPARATION

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

9.2. Microplate preparation

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

9.3. Washing Solution preparation

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

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

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

10. ASSAY PROCEDURE

10.1. Put the desired number of strips into the frame based on the number of test samples and 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 **50 µL of Calibrators and Control Serum as well as 50 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

During performing several independent series of tests, Calibrators and Control 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 **90 minutes at +37°C**.

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

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

10.8. Read the optical density (OD) of the wells at 450 nm 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 17-OH-progesteron concentration in the calibrators nmol/L, (y) – OD versus 17-OH-progesteron 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 17-OH-progesterone in tested samples from the calibration curve.

11. TEST VALIDITY

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

12. EXPECTED VALUES

12.1. Therapeutical consequences should not be based on the results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for 17-OH-progesterone. Based on data obtained by XEMA LLC, the following normal range is recommended (see below).

NOTE: values of 17-OH-progesterone concentrations in the tested samples that are below the LoD (0.12 nmol/L) and also exceed the value of the upper calibrator (100 nmol/L) should be provided in the following form: «the 17-OH-progesterone concentration of tested sample X is «lower than 0.12 nmol/L» or «higher than 100 nmol/L».

12.2. The calibrators concentration values of the 17-OH-progesterone EIA kit are expressed in nmol/L. To calculate concentrations in ng/ml, the received concentration value in nmol/L shall be multiplied by 0.32.

1 nmol/L = 0.32 ng/mL

Sex, age	Units, nmol/L		Units alternative, ng/mL	
	Lower limit	Upper limit	Lower limit	Upper limit
newborn	0.7	2.3	0.23	0.75
children	0.1	2.7	0.03	0.89
Males				
puberty	0.2	5.3	0.07	1.74
postpuberty	0.9	6.0	0.30	1.97
Females				
puberty	0.1	7.0	0.03	2.3
postpuberty	0.2	8.7	0.07	2.85
pregnancy	2.0	12	0.66	3.93
Menstrual cycle				
follicular phase	0.2	2.4	0.07	0.79
luteinic phase	0.9	8.7	0.3	2.85
post menopausal	0.12	7.0	0.04	2.3

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1. Precision of Measurement

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

Sample	Concentration, nmol/L	CV, %
1	17.15	4.3
2	6.04	5.4

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

Sample	Concentration, nmol/L	CV, %
1	18.18	3.2
2	5.97	6.0

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

Sample	Concentration 1, nmol/L	Concentration 2, nmol/L	Concentration 3, nmol/L	CV, %
1	15.77	18.18	19	9.5

2	6.09	5.98	4.68	13.8

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 17-OH-progesterone concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 0.5–100 nmol/L ±10%.

13.1.4. Analytical sensitivity

Limit of detection (LoD) – the lowest 17-OH-progesterone concentration in the serum or plasma sample that is detected by the 17-OH-progesterone 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 17-OH-progesterone 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 17-OH-progesterone with other analytes is shown in the table:

Analyte	Cross-reactivity, %
11-Deoxycortisol	2.3
Progesterone	< 1.0

other steroids	< 0.1

14. REFERENCES

1. Abraham, G.E., R.S. Swerdloff, D. Tulchinsky et al: Radioimmunoassay of plasma 17- hydroxyprogesterone. J. Clin. Endocrinol. Metab. 33:42, 1971.
2. Chrousos, G.P., D. L. Loriaux, D.L. Mann, et al: Late onset 21- hydroxylase deficiency mimicking idiopathic hirsutism or polycystic avarian disease. Annals Intern. Med. 96:143, 1982.
3. Buster, J.E., R.J. Chang, D.L. Preston, et al: Interrelationships of circulating maternal steroids; progesterone, 16ahydroxyprogesterone, 17a-hydroxyprogesterone, 20a-dihydroprogesterone, gamma-5-pregnolonone, gamma-5-pregnolonone-sulfate, gamma-5-pregnolone-sulfate and 17-hydroxy gamma-5-pregnolonone, J. Clin. Endocrinol. Metab. 48:133, 1979.
4. New, M.I., B. Dupont, S. Pang, et al: An update on congenital adrenal hyperplasia. Recent Progress in Hormone Research, 37:105, 1981.
5. Pang S., J. Hotchkiss, A. Drash, et al: Micro filter paper method for 17ahydroxyprogesterone radioimmunoassay: Its application for rapid screening for congenital adrenal hyperplasia. J. Clin. Endocrinol. Metab., 45:1003, 1977.
6. Lobo, R.A., U. Goebelsmann: Adult manifestation of congenital adrenal hyperplasia due to incomplete 21-hydroxylase deficiency mimicking polycystic ovarian disease. Am. J. Obstet. Gynecol., 138:720, 1980.
7. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
8. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
9. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81).

SAMPLES IDENTIFICATION PLAN

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

LOT _____ DATE _____

Instruction version/date: 2024.07

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 _____

K217IE

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

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

+38 044 294-69-78

or write to:

qa@xema.com.ua

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www.xema.com.ua

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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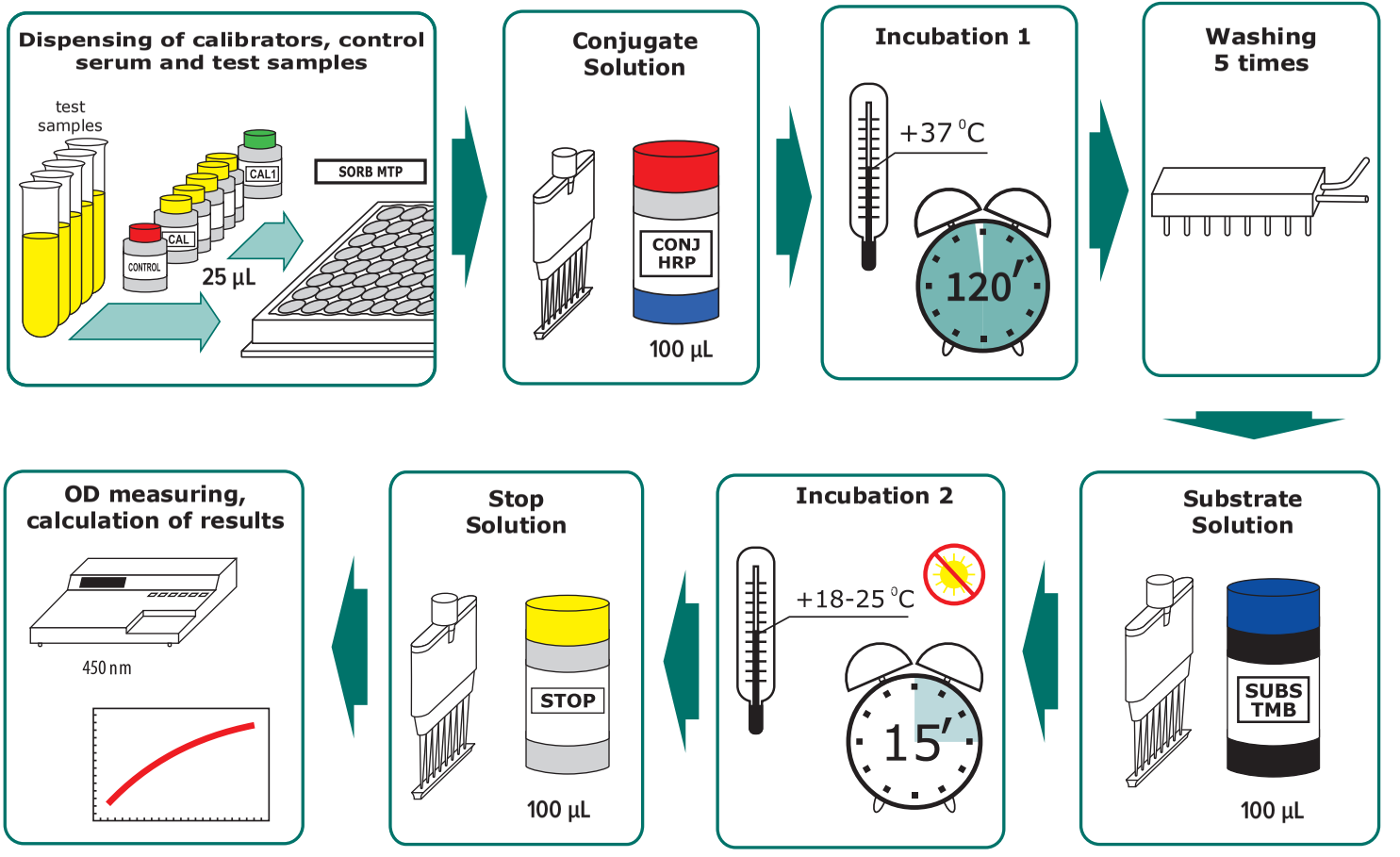
tel.:+ 49 911 931 639 67
E-mail: info@polmed.de
www.polmed.de

ASSAY PROCEDURE



K219

CONTENT

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

1. INTENDED USE

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

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

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

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

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

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

3. TEST PRINCIPLE

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

The analysis procedure includes two stages of incubation:

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

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

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

4. KIT COMPONENTS

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

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

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

6. WARNING AND PRECAUTIONS

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

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

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

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

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

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

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

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

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

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

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

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

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

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

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

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

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

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

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

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

8.1. Transportation

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

8.2. Storage

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

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

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

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

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

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

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

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

8.3. Disposal

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

9. REAGENTS PREPARATION

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

9.2. Microplate preparation

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

9.3. Washing Solution preparation

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

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

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

10. ASSAY PROCEDURE

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

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

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

Scheme of introduction of samples

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

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

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

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

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

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

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

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

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

11. TEST VALIDITY

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

12. EXPECTED VALUES

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

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

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

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

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

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

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

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

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

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

13.1.2 Trueness

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

13.1.3 Linearity

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

13.1.4 Analytical sensitivity

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

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

13.1.5 Analytical specificity

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

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

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

14. REFERENCES

1. Tietz, N.W. Textbook of Clinical Chemistry. Saunders, 1986.
2. Joshi, U. M., et al. Steroids 34 (1) 35 (1979).
3. Turkes, A., et al. J Endocrinol. 81 (2) P165 (1979).
4. Ismail, A. A., Niswender, G. D. Midgley, A. R. J. Clin. Endocr. Metab. 34, 177 - 184 (1972).
5. Rajkowski,K. M.,Cittanova N.,Desfosses, B.and Jayle, M.F. Steroids 29 no 5 1977 6. Widsdom G. B. Clin. Chem. 22/8, 1243 - 1255 (1976).
6. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
7. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
8. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

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

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

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

LOT _____ DATE _____

	Manufacturer
	*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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