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
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STATEMENT

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

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

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

Date: 06.09.2023

Signature:

Director Xema LLC
Oleksandra Zavaliei

Digitally signed by Dolgopol Iulian
Date: 2025.10.03 10:47:01 EEST
Reason: MoldSign Signature
Location: Moldova
MOLDOVA EUROPEANĂ

EC DECLARATION OF CONFORMITY
EU- KONFORMITATSERKLARUNG

98/79/EC in connection with article 110(3) IVDR
98/79/EG in Verbindung mit Artikel 110 (3) IVDR

No.**XEMA_LLC- DC-01/2025**

Manufacturer: Hersteller:	**XEMA LLC** UKRAINE, 03179 KYIV Akademika Yefremova St. 23 Tel./Fax: +38 044 294-69-78 Email: qa@xema.com.ua www.xema.com.ua	**Single registration number (SRN)** Einmalige Registrierungsnummer: **UA-MF-000032959**
EC Authorized Representative: EU-Bevollmächtigte:	**Polmed.de Beata Rozwadowska** Fichtenstr. 12A 90763 Fürth Germany/Deutschland Tel: +49 911 931 639 67 www.polmed.de	**Single registration number (SRN)** Einmalige Registrierungsnummer: **DE-AR-000006947**
Product name: Produktbezeichnung:	**see annex / siehe Anhang**	
Classification (Risk class): Klassifizierung (Risikoklasse):	**Common/ Other IVD** Sonstige IVD-Produkte	
Conformity assessment procedure: Konformitatsbewertungsverfahren:	**Appendix III (points 1-5) of Directive 98/79/EC** Anhang III (Nr. 1-5) der Richtlinie 98/79/EG	

Standards applied/Angewandte Normen:

ISO 9000:2015	Quality management systems — Fundamentals and vocabulary
ISO 19011:2018	Guidelines for auditing management systems
ISO 13485:2016	Medical devices — Quality management systems — Requirements for regulatory purposes
ISO 14971:2019	Medical devices. Application of risk management to medical devices
EN ISO 15223-1:2021	Medical devices - Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements (ISO 15223-1:2021)
EN ISO 18113-1:2024	In vitro diagnostic medical devices – Information supplied by the manufacturer (labelling) – Part 1: Terms, definitions and general requirements (ISO 18113-1:2022)
EN ISO 18113-2:2024	In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 2: In vitro diagnostic reagents for professional use (ISO 18113-2:2022)

We hereby declare under our the sole responsibility, that the devices listed in the Annex meet all applicable provision of the EU (IVDD). The procedure according to Annex III of the Regulation (EU) 2017/746 (IVDR) has been followed.

Wir erklären hiermit in unserer alleinigen Verantwortung, dass die im Anhang genannten Produkte alle anwendbaren Bestimmungen der EG-Richtlinie 98/79/WG (IVDD)entsprechen. Das Verfahren gemäß Anhang III der Verordnung (EU) 2017/746 (IVDR) wurde eingehalten.

If the product is changed/transformed without the consent of the undersigned, this declaration becomes invalid with regard to the modified/converted product.

Wenn das Produkt ohne Zustimmung des Unterzeichneten geändert/transformiert wird, wird diese Erklärung in Bezug auf das modifizierte/konvertierte Produkt ungültig.

Validity/Gültigkeit: **until**/ bis: **31.12.2028**

Signature/ Unterschrift:
Name: **Oleksandra Zavaliei**
Position: Director "XEMA LLC"

Place and date of issue:
Ort und Datum der Ausgabe: Kyiv
26.05. 2025

EC DECLARATION OF CONFORMITY
EU- KONFORMITATSERKLARUNG

98/79/EC in connection with article 110(3) IVDR
98/79/EG in Verbindung mit Artikel 110 (3) IVDR

No.**XEMA_LLC- DC-01/2025**

Annex to Declaration of conformity
Anhang zur Konformitätserklärung

Product list /Produktliste

#	**Nomenclature term** Nomenklaturbezeichnung **EDMA**	**Cat. #** Katalog-Nr.:	**Name of device** Produktbezeichnung	**Nomenclature Code** Nomenklaturcode **EDMA**	**Category IVDD** Kategorie IVDD
1.	ASPERGILLUS	K021	GalMAg EIA	15-06-01-01-00	other
2.	HSV IgG	K104	HSV 1/2 IgG EIA	15-04-03-05-00	other
3.	HSV IgM	K104M	HSV 1, 2 IgM EIA	15-04-03-06-00	other
4.	HSV 2 IgG	K104B	HSV 2 IgG EIA	15-04-03-11-00	other
5.	MYCOPLASMA ANTIBODY ASSAYS	K106	Mycoplasma IgG EIA	15-01-08-03-00	other
6.	SYPHILIS ANTIBODY ASSAYS TOTAL	K111	anti-Treponema pallidum EIA	15-01-03-03-00	other
7.	SYPHILIS ANTIBODY IGG	K111G	Treponema pallidum IgG EIA	15-01-03-05-00	other
8.	H. PYLORI ANTIBODY ASSAYS	K119G	Helicobacter pylori IgG EIA	15-01-04-03-00	other
9.	OTHER OTHER BACTERIOLOGY IMMUNOASSAY	K126	Ureaplasma IgG EIA	15-01-90-90-00	other
10.	THYROID PEROXIDASE (INCL. MICROSOMAL) ANTIBODIES	K131	aTPO EIA	12-10-03-01-00	other
11.	THYROGLOBULIN AUTOANTIBODIES	K132	aTG EIA	12-10-03-04-00	other
12.	MPO ANCA	K133	aMPO EIA	12-10-90-09-00	other
13.	TISSUE TRANSGLUTAMINASE ANTIBODIES	K160 K161	anti-TGlu IgG EIA anti-TGlu IgA EIA	12-10-90-21-00	other
14.	GIARDIA LAMBLIA	K171	anti-Giardia lamblia EIA	15-05-10-08-00	other
15.	OTHER PARASITOLOGY	K174	Ascaris IgG EIA	15-05-10-90-00	other
16.	ECHINOCOCCUS	K175	Echinococcus IgG EIA	15-05-10-04-00	other
17.	DISTOMATOSIS	K176	Opisthorchis IgG EIA	15-05-10-03-00	other
18.	GLIADIN ANTIBODIES	K180 K181	Gliadin IgG EIA Gliadin IgA EIA	12-10-90-06-00	other
19.	IMMUNOGLOBULIN E – TOTAL	K200	Total IgE EIA	12-02-01-02-00	other
20.	THYROID STIMULATING HORMONE	K201	TSH EIA	12-04-01-11-00	other
21.	LUTEINISING HORMONE	K202	LH EIA	12-05-01-05-00	other
22.	FOLLICLE STIMULATING HORMONE	K203	FSH EIA	12-05-01-04-00	other
23.	HUMAN GROWTH HORMONE	K204	GH EIA	12-06-04-02-00	other
24.	HUMAN CHORIONIC GONADOTROPIN TOTAL	K205	hCG EIA	12-05-02-05-00	other
25.	PROLACTIN	K206	Prolactin EIA	12-05-01-08-00	other
26.	PROGESTERONE	K207	Progesterone EIA	12-05-01-06-00	other
27.	ESTRADIOL	K208	Estradiol EIA	12-05-01-03-00	other
28.	TESTOSTERONE (WITH DEHYDRO AND FREE TESTOSTERONE)	K209	Testosterone EIA	12-05-01-10-00	other
29.	CORTISOL	K210	Cortisol EIA	12-06-02-04-00	other
30.	TRIIODOTHYRONINE	K211	T3 EIA	12-04-01-05-00	other
31.	THYROXINE	K212	T4 EIA	12-04-01-07-00	other
32.	FREE TRIIODOTHYRONINE	K213	fT3 EIA	12-04-01-01-00	other
33.	FREE THYROXINE	K214	fT4 EIA	12-04-01-02-00	other
34.	DEHYDRO-EPIANDROSTERONE SULPHATE (INCL. DHEA)	K215	DHEAS EIA	12-05-01-02-00	other
35.	17 OH PROGESTERONE	K217	17-OH-progesterone EIA	12-05-01-07-00	other
36.	ESTRIOL	K218	free Estriol EIA	12-05-02-02-00	other
37.	TESTOSTERONE (WITH DEHYDRO AND FREE TESTOSTERONE)	K219	free Testosterone EIA	12-05-01-10-00	other
38.	CANCER ANTIGEN 125	K222	CA 125 EIA	12-03-01-06-00	other

#	Nomenclature term EDMA	Cat. #	Name of device	Nomenclature Code EDMA	Category IVDD
42.	CANCER ANTIGEN 15-3	K226	CA 15-3 (M12) EIA	12-03-01-02-00	other
43.	OTHER OTHER TUMOUR MARKERS	K232	Thyroglobulin EIA	12-03-90-90-00	other
44.	ß HUMAN CHORIONIC GONADOTROPIN (INCL. SUBUNIT)	K235	free β-HCG EIA	12-05-02-06-00	other
45.	CYFRA 21-1	K236	CYFRA 21-1 EIA	12-05-02-10-00	other
46.	SQUAMOUS CELL CARCINOMA ANTIGEN	K237	SCC (A) EIA	12-03-01-35-00	other
47.	PREGNANCY ASSOCIATED PLASMA PROTEIN - A (DOWNS)	K238	PAPP-A EIA	12-05-02-10-00	other
48.	OTHER OTHER TUMOUR MARKERS	K239	HE4 EIA	12-03-90-90-00	other
49.	CANCER ANTIGEN 242	K243	CA242 EIA	12-03-01-08-00	other
50.	OTHER PREGNANCY TESTING HORMONES	K245	AMH EIA	12-05-02-90-00	other
51.	HUMAN PLACENTAL LACTOGEN HPL	K246	Placental lactogen EIA	12-05-02-07-00	other
52.	C-REACTIVE PROTEIN	K250	CRP EIA	12-11-01-09-00	other
53.	C-PEPTIDE	K267C	C-peptide EIA	12-06-01-01-00	other
54.	INSULIN	K267N	Insulin EIA	12-06-01-03-00	other
55.	SEX HORMONE BINDING GLOBULIN	K268	SHBG EIA	12-05-01-09-00	other
56.	TROPONIN (T + I)	K291	Troponin I EIA	12-13-01-07-00	other
57.	LYME ANTIBODY IGG	K118G	Borelia burgdorferi IgG EIA	15-01-06-05-00	other
58.	LYME ANTIBODY IGM	K118M	Borelia burgdorferi IgM EIA	15-01-06-06-00	other
59.	EBV ANTIBODIES	K108V K108VM K108N	Epstein-Barr virus VCA IgG EIA Epstein-Barr virus VCA IgM EIA Epstein-Barr virus EBNA IgG EIA	15-04-04-04-00	other

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.

Instruction for use
A solid-phase enzyme immunoassay kit
for the quantitative determination of
antigen CYFRA 21-1 in human serum or plasma

CYFRA 21-1 EIA

Catalogue number REF **K236**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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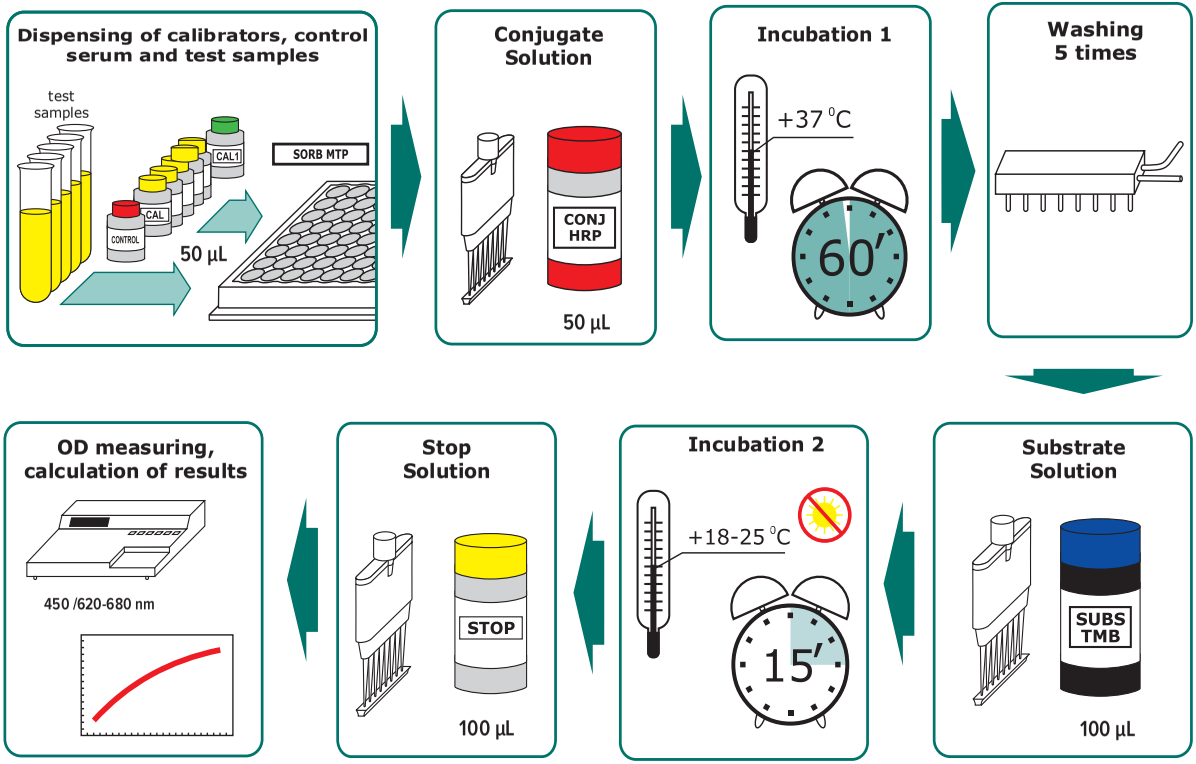
ASSAY PROCEDURE



K236

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of antigen CYFRA 21-1 in human serum or plasma
CYFRA 21-1 EIA

1. INTENDED USE

The CYFRA 21-1 EIA kit is an enzyme immunoassay, intended for the quantitative determination of antigen CYFRA 21-1 in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

The CYFRA 21-1 antigen is a fragment of cytokeratin 19, which is formed as a result of proteolysis and, unlike the main cytokeratin structure, is able to change into a soluble form and enter the systemic bloodstream.

The precursor molecule of the CYFRA 21-1 antigen - cytokeratin 19 - is expressed in all normal tissues, but a particularly high level of expression is observed in lung or bladder wall tumor cells.

Increased content of CYFRA 21-1 is observed in the blood of patients with lung tumors (mainly squamous cell carcinoma, less often adenocarcinoma and other histological forms) and bladder tumors. Determination of the level of the CYFRA 21-1 antigen is useful for monitoring the effectiveness of treatment and monitoring the course of these tumors; however, the results of the measurement of the CYFRA 21-1 antigen should always be interpreted in conjunction with the results of other research methods and clinical data.

3. TEST PRINCIPLE

The determination of the CYFRA 21-1 is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to soluble cytokeratin 8/19 (CYFRA 21-1). Second antibodies – murine monoclonal antibodies to human CYFRA 21-1 conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage CYFRA 21-1 from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized CYFRA 21-1;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured CYFRA 21-1in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density on the content of CYFRA 21-1in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P236Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to CYFRA 21-1; ready to use
C236Z	CAL 1	**Calibrator C1**	2 mL	1	Solution based on phosphate buffer (pH 7.2-7.4), free of CYFRA 21-1, with preservative, ready to use (colourless liquid)
C236Z	CAL 2-5	**Calibrators**	0.8 mL	4	Solution based on phosphate buffer (pH 7.2-7.4), containing 3; 10; 25 and 50 ng/mL of CYFRA 21-1, with preservative, ready to use (red liquids)
Q236Z	CONTROL	**Control Serum**	0.8 mL	1	Solution based on human serum, containing of known CYFRA 21-1 content, with preservative, ready to use (colourless liquid)
T236Z	CONJ HRP	**Conjugate Solution**	6 mL	1	Solution of murine monocnoclonal antibodies to CYFRA 21-1 conjugated to the horseradish peroxidase; ready to use (red liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

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

6. WARNING AND PRECAUTIONS

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

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

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

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

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

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

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

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

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

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

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

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

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

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

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

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

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

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

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

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

8.1. Transportation

The CYFRA 21-1 EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The CYFRA 21-1 EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

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

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

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

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

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

8.3. Disposal

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

9. REAGENTS PREPARATION

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

9.2. Microplate preparation

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

9.3. Washing Solution preparation

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

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

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

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 50 ng/mL, additionally dilute this sample accordingly, using (Calibrator C1). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

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

Do not dilute Control Serum and Calibrators!

10. ASSAY PROCEDURE

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

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

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

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

10.4 Dispense **50 µL of Conjugate Solution** to all wells.

Scheme of introduction of samples

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

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

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

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

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

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

10.10 Plot a calibration curve in linear coordinates: (x) is the CYFRA 21-1 concentration in the calibrators ng/mL, (y) – OD versus CYFRA 21-1 concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.11 Determine the corresponding concentration of CYFRA 21-1 in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

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

12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for CYFRA 21-1. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered antimouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

NOTE: values of CYFRA 21-1 concentrations in the tested samples that are below the LoD (0.5 ng/mL) and also exceed the value of the upper Calibrator (50 ng/mL) should be provided in the following form : «the CYFRA 21-1 concentration of tested sample X is «lower than 0.5 ng/mL» or «higher than 50 ng/mL».

Sex, age	Units, ng/mL	
	Lower limit	Upper limit
Healthy donors	-	3.0

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

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

Sample	Concentration, ng/mL	CV, %
1	12.3	6.2
2	25	3.3

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

Sample	Concentration, ng/mL	CV, %
1	12.27	4.3
2	25.89	5.2

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

Sample	Concentration1, ng/mL	Concentration2, ng/mL	Concentration3, ng/mL	CV, %
1	12.32	12.02	12.81	5.2
2	25.02	25.6	26.0	2.9

13.1.2 Trueness

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

13.1.3 Linearity

Linearity was determined using sera samples with known CYFRA 21-1 concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 3-25 ng/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest CYFRA 21-1 concentration in the serum or plasma sample that is detected by the CYFRA 21-1 EIA kit is no lower than 0.5 ng/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for CYFRA 21-1 EIA kit is 3 ng/mL.

13.1.5 Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 50 ng/mL.

13.1.6 Analytical specificity

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

The cross-reactivity of CYFRA 21-1 with other analytes is shown in the table:

Analyte	Cross-reactivity, %
CA 15-3	<0.1
CA 125	<0.1
CA 19-9	<0.1
AFP	<0.1
PSA	<0.1

14. REFERENCES

1. Petra Stieber CYFRA 21-1 (Cytokeratin-19-Fragment), in: Lothar Thomas, Labor und Diagnose, TH Brooks, Frankfurt, Germany
2. J-L Pujol, O Molinier, W Ebert et al. (2004) British Journal of Cancer 90 (11):2097-2105
3. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
4. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
5. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

SAMPLES IDENTIFICATION PLAN

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XEMA

SAMPLES IDENTIFICATION PLAN

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LOT _____ DATE _____

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

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

+38 044 294-69-78

or write to:

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of carbohydrate antigen 242 in human serum or plasma

CA 242 EIA

Catalogue number REF **K243**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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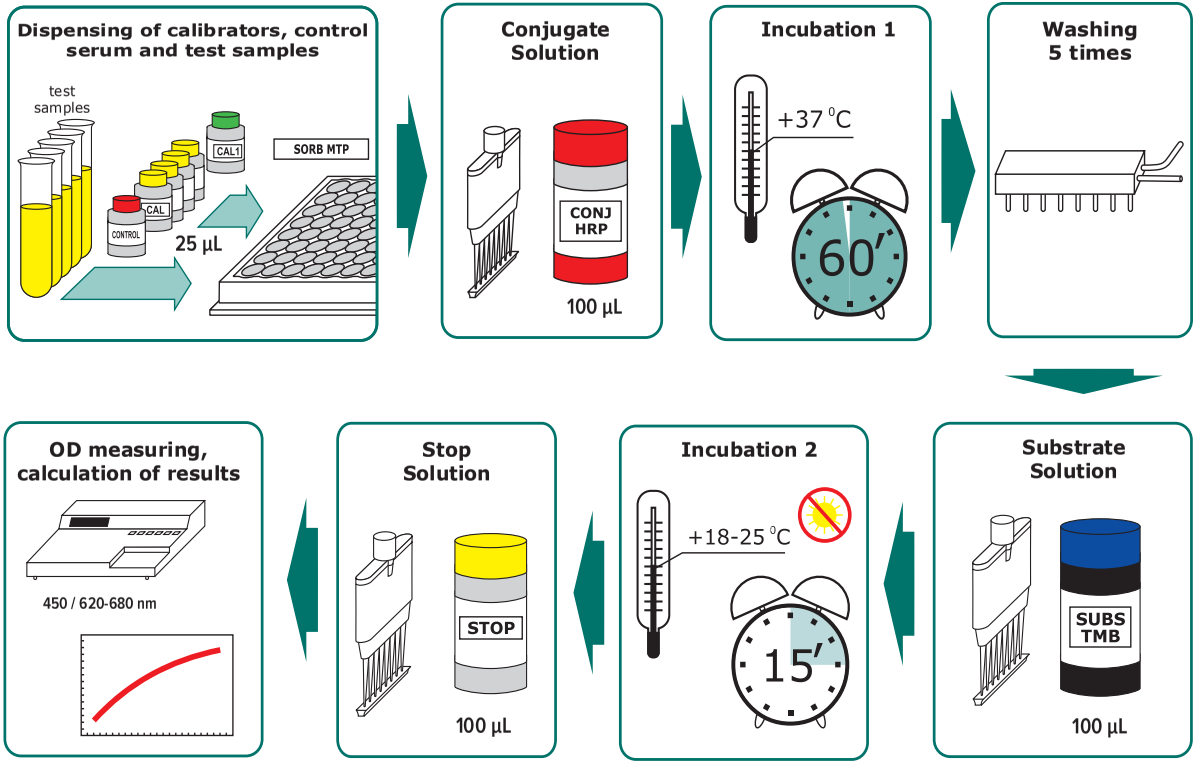
ASSAY PROCEDURE



K243

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of carbohydrate antigen 242 in human serum or plasma
CA 242 EIA

1. INTENDED USE

The CA 242 EIA kit is an enzyme immunoassay, intended for the quantitative determination of carbohydrate antigen 242 in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

The carbohydtrate antigen CA 242 is one of the most advanced markers of gastrointestinal cancer. CA 242 is found on cells of colonal mucosa as well as on apical part of cells lining pancreatic ducts.

CA 242 is one of the most important markers used in oncology. For differential diagnostics between pancreatic cancer (PC) and chronic pancreatitis, diagnostic specificity of CA 242 is 1.4 fold higher than that of CA 19-9. In patients with PC, a positive prognostic value of CA 242 determination is higher than that of CA 19-9 at any stage of the disease.

3. TEST PRINCIPLE

The determination of the CA 242 is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to CA 242/CA 19-9. Second antibodies – murine monoclonal antibodies to human CA 242 conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage CA 242 from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized CA 242;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3’,5,5’-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured CA 242 in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density on the content of CA 242 in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P223Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to CA 242/CA 19-9; ready to use
C243Z	CAL 1	**Calibrator C1**	2 mL	1	Solution based on tris buffer (pH 7.2-7.4), free of CA 242, with preservative, ready to use (colourless liquid)
C243Z	CAL 2-5	**Calibrators**	0.5 mL	4	Solutions based on tris buffer (pH 7.2-7.4), containing 15; 50; 100 and 200 U/mL of CA 242, with preservative, ready to use (blue liquids)
Q243Z	CONTROL	**Control Serum**	0.5 mL	1	Solution based on human serum, containing of known CA 242 content, with preservative, ready to use (colourless liquid)
T243Z	CONJ HRP	**Conjugate Solution**	14 mL	1	Solution of murine monoclonal antibodies to human CA 242 conjugated to the horseradish peroxidase; ready to use (red liquid)
R055Z	SUBS TMB	**Substrate Solution**	14 mL	1	Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	14 mL	1	5.0% solution of sulphuric acid; ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

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

6. WARNING AND PRECAUTIONS

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

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

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

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

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

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

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

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

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

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

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

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

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

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

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

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

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

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

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

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

8.1. Transportation

The CA 242 EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The CA 242 EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

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

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

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

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

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

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

8.3. Disposal

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

9. REAGENTS PREPARATION

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

9.2. Microplate preparation

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

9.3. Washing Solution preparation

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

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

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

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 200 U/mL, additionally dilute this sample accordingly, using (Calibrator C1). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

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

10. ASSAY PROCEDURE

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

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

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

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

Scheme of introduction of samples

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

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

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

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

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

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

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

10.13 Plot a calibration curve in linear coordinates: (x) is the CA 242 concentration in the calibrators U/mL, (y) – OD versus CA 242 concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.14 Determine the corresponding concentration of CA 242 in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

11. TEST VALIDITY

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

12. EXPECTED VALUES

12.1. Therapeutical consequences should not be based on results of IVD methods alone - all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for CA 242. Based on data obtained by XEMA LLC, the following normal range is recommended (see below).

NOTE: values of CA 242 concentrations in the tested samples that are below the LoD (0.5 U/mL) and also exceed the value of the upper Calibrator (200 U/mL) should be provided in the following form : «the CA 242 concentration of tested sample X is «lower than 0.5 U/mL» or «higher than 200 U/mL».

Sex, age	Units, U/mL	
	Lower limit	Upper limit
Males	-	20
Females	-	20

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

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

Sample	Concentration, U/mL	CV, %
1	10.12	3.2
2	53.64	2.8

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

Sample	Concentration, U/mL	CV, %
1	10.27	7.0
2	53.87	6.1

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

Sample	Concentration1, U/mL	Concentration2, U/mL	Concentration3, U/mL	CV, %
1	10.32	10.02	10.81	3.8
2	53.71	53.56	54.32	0.6

13.1.2 Trueness

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

13.1.3 Linearity

Linearity was determined using sera samples with known CA 242 concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 15-100 U/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest CA 242 concentration in the serum or plasma sample that is detected by the CA 242 EIA kit is no lower than 0.5 U/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for CA 242 EIA kit is 15 U/mL.

13.1.5 Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 200 U/mL.

13.1.6 Analytical specificity

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

The cross-reactivity of CA 242 with other analytes is shown in the table:

Analyte	Cross-reactivity, %
CEA	<0.1
CA 15-3	<0.1

14. REFERENCES

1. Rana S, Dutta U, Kochhar R, Rana SV, Gupta R, Pal R, Jain K, Srinivasan R, Nagi B, Nain CK, Singh K. Evaluation of CA 242 as a tumor marker in gallbladder cancer. J Gastrointest Cancer. 2012 Jun;43(2):267-71.
2. Tian SB, Yu JC, Kang WM, Ma ZQ, Ye X, Cao ZJ, Yan C. Combined detection of CEA, CA 19-9, CA 242 and CA 50 in the diagnosis and prognosis of resectable gastric cancer. Asian Pac J Cancer Prev. 2014;15(15):6295-300.
3. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
4. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
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SAMPLES IDENTIFICATION PLAN

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LOT _____ DATE _____

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

+38 044 294-69-78

or write to:

qa@xema.com.ua

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Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of carbohydrate antigen 72-4 in human serum or plasma

CA 72-4 EIA

Catalogue number REF **K244**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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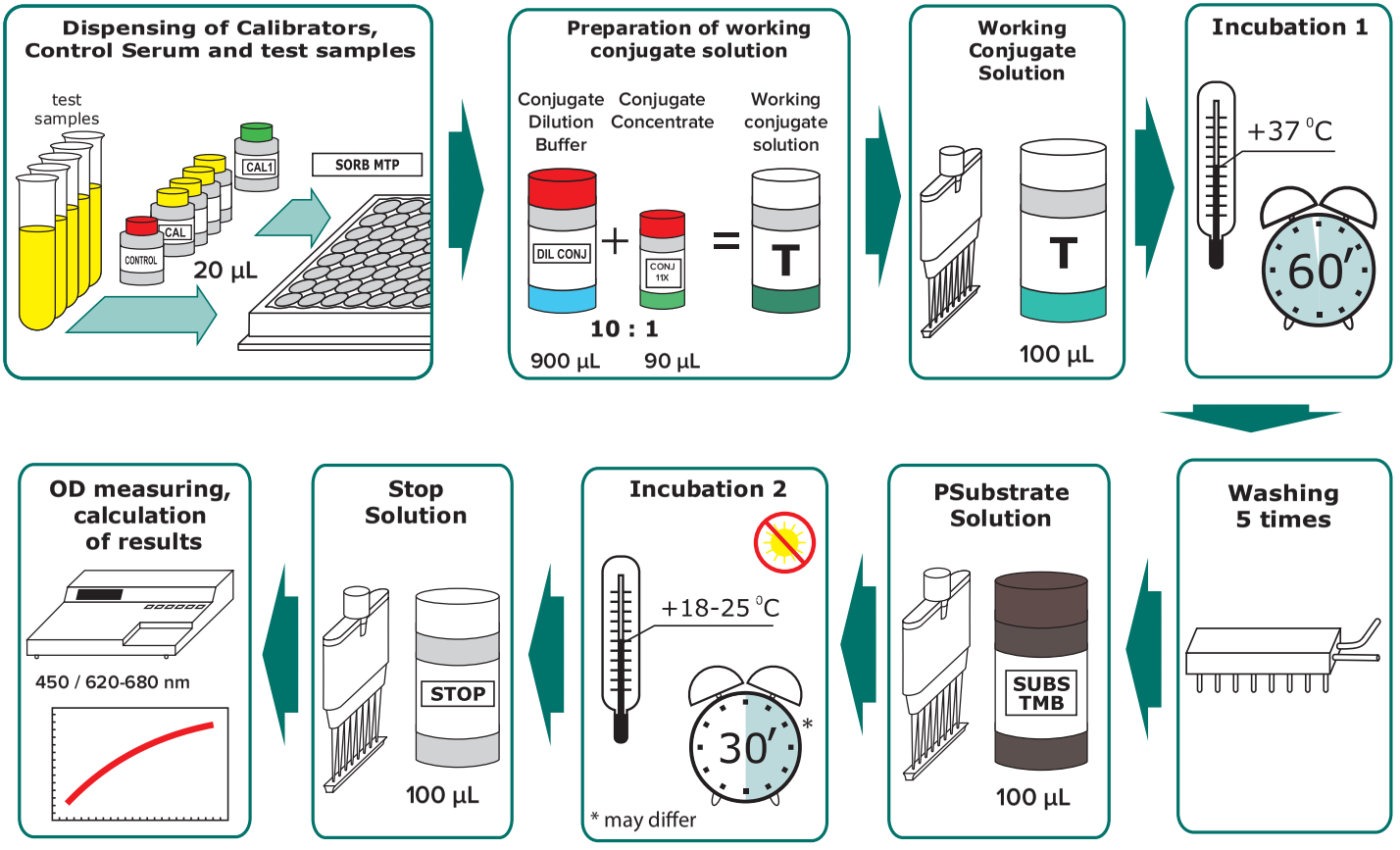
ASSAY PROCEDURE



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

CONTENT

Instruction for use
A solid-phase enzyme immunoassay kit for the quantitative determination of carbohydrate antigen 72-4 in human serum or plasma
CA 72-4 EIA

1. INTENDED USE

The CA 72-4 EIA kit is an enzyme immunoassay, intended for the quantitative determination of carbohydrate antigen 72-4 in human serum or plasma.

Determination of CA 72-4 antigen concentration in serum (plasma) is used as an auxiliary method of early diagnosis, monitoring the effectiveness of therapy in malignant tumors of glandular tissue, such as gastric carcinoma, colon or ovarian cancer, for all population groups.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

CA 72-4, or a carbohydrate antigen 72-4, is a high MM (230-1000 kD) antigen (epitope) associated to gastric and ovarian cancer as well as some other malignancies and not expressed in noticeable quantities in tissues of healthy adult individuals.

Quantitative determination of CA 72-4 in serum or plasma is helpful (particularly, in combination with CA 19-9 – see XEMA LLC, Cat.# K223) for monitoring of gastric cancer and its therapy, while combined determination of CA 72-4 and CA 125 (see XEMA LLC, Cat.# K222) is used for monitoring of ovarian cancer.

Elevated levels of CA 72-4 are often seen in adenocarcinomas of the gastro-intestinal tract, ovaries (mucinous type) and lungs. Besides, raised CA 72-4 is sometimes also seen in patients with benign pathology (chronic inflammation, cysts, fibrosis). That is why, results of CA 72-4 determination should always be interpreted in conjunction with other laboratory and clinical data.

Functional purpose. Determination of the concentration of CA 72-4 antigen in serum (plasma) is used as an auxiliary method for early diagnosis, monitoring the effectiveness of therapy for malignant glandular tumours, such as gastric carcinoma, colon or ovarian cancer, for all population groups.

3. TEST PRINCIPLE

The determination of carbohydrate antigen 72-4 is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to human CA 72-4. Second antibodies – murine monoclonal antibodies to human CA 72-4 conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage CA 72-4 from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized CA 72-4;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured CA 72-4 in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density on the content of CA 72-4 in the calibration samples.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P244Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with murine monoclonal antibodies to human CA 72-4, ready to use
C244Z	CAL 1	**Calibrator C1**	0.5 mL	1	Solution based on human serum, free of CA 72-4, with preservative, ready to use (yellow liquid)
C244Z	CAL 2-5	**Calibrators**	0.5 mL	4	Solutions based on human serum, containing 5; 15; 50 and 200 U/mL of CA 72-4, with preservative, ready to use (blue liquids)
Q244Z	CONTROL	**Control Serum**	0.5 mL	1	Solution based on human serum, containing of known CA 72-4 content, with preservative, ready to use (yellow liquid)
T244XZ	CONJ 11X	**Conjugate Concentrate**	1.2 mL	1	Solution of murine monocnoclonal antibodies to human CA 72-4 conjugated to the horseradish peroxidase, 11x concentrate (green liquid)
ST244Z	DIL CONJ	**Conjugate Dilution Buffer**	12 mL	1	Buffer solution with detergent, ready to use (blue liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution, ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	22 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid, ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (1 pcs.)					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

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

6. WARNING AND PRECAUTIONS

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

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

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

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

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

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

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

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

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

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

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

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

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

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

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

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

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

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

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

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

8.1. Transportation

The CA 72-4 EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The CA 72-4 EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

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

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

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

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

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

8.3. Disposal

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

9. REAGENTS PREPARATION

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

9.2. Microplate preparation

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

9.3. Washing Solution preparation

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

9.4. Working conjugate solution preparation

Prepare in a different container a working conjugate solution by 11 dilutions of Conjugate Concentrate in Conjugate Dilution Buffer (eg, 90 µL of concentrate + 900 µL of Conjugate Dilution Buffer). In the case of partial use of the kit, take the necessary amount of Conjugate Concentrate and dilute it 11 times with Conjugate Dilution Buffer, since the working conjugate solution in a diluted form is not stored for a long time.

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

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	1.8	3.6	5.4	7.2	9	10.8	12.6	14.4	16.2	18	19.8	22
Volume of water, mL	45	90	135	180	225	270	315	360	405	450	495	550
Volume of Conjugate Concentrate, mL	0.09	0.18	0.27	0.36	0.45	0.54	0.63	0.72	0.81	0.9	0.99	1.08
Volume of Conjugate Dilution Buffer, mL	0.9	1.8	2.7	3.6	4.5	5.4	6.3	7.2	8.1	9	9.9	10.8

10. ASSAY PROCEDURE

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

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

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

Scheme of introduction of samples

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

10.3. Dispense **100 µL of Working conjugate solution** to all wells (see 9.4).

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

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

10.6. Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 30 minutes.**
The incubation time can be varied depending on the intensity of the blue colour development.

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

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

10.9. Plot a calibration curve in linear coordinates: (x) is the concentration of CA 72-4 in the Calibrators U/mL, (y) – OD versus concentration of CA 72-4 (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. For the algorithm calculation (approximation) of the calibration curve, using the interval (segment-linear, point-to-point) method is recommended.

10.10. Determine the corresponding concentration of CA 72-4 in tested samples from the calibration curve.

11. TEST VALIDITY

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

12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for CA 74-2. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered antimouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

NOTE: values of CA 74-2 concentrations in the tested samples that are below the LoD (0.3 U/mL) and also exceed the value of the upper calibrator (200 U/mL) should be provided in the following form : «the CA 74-2 concentration of tested sample X is «lower than 0.3 U/mL» or «higher than 200 U/mL».

Sex, age	Units, U/mL	
	Lower limit	Upper limit
Healthy donors	-	6.0

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1. Precision of Measurement

Reproducibility. The coefficient of variation of determining the content of CA 72-4 in the same sample of blood serum (plasma) using the kit CA 72-4 EIA does not exceed 10%.

13.1.2. Trueness

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

13.1.3. Linearity

Linearity was determined using sera samples with known CA 72-4 concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 5–200 U/mL ±10%.

13.1.4. Analytical sensitivity

Limit of detection (LoD) – the lowest CA 72-4 concentration in the serum or plasma sample that is detected by the CA 72-4 EIA kit is no lower than 0.3 U/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for CA 72-4 EIA kit is 5 U/mL.

13.1.5. Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 20000 U/mL.

13.1.6. Analytical specificity

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

The cross-reactivity of CA 74-2 with other analytes is shown in the table:

Analyte	Cross-reactivity, %
CEA	<0.1
CA 125	<0.1
CA 19-9	<0.1

14. REFERENCES

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2. Ian J. Jacobs and Usha Menon – Progress and Challenges in Screening for Early Detection of Ovarian Cancer. Mol. Cell. Proteomics, Apr 2004; 3: 355 – 366.
3. R Hamazoe, M Maeta, T Matsui, S Shibata, S Shiota, and N Kaibara – CA72-4 compared with carcinoembryonic antigen as a tumour marker for gastric cancer. Eur J Cancer, Jan 1992; 28A(8-9): 1351-4
4. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
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SAMPLES IDENTIFICATION PLAN

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

LOT _____ DATE _____

SAMPLES IDENTIFICATION PLAN

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

LOT _____ DATE _____

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

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

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