Instructions for use

Cardiolipin IgM ELISA

Enzyme immunoassay for the qualitative and quantitative determination of IgM antibodies against cardiolipin in human serum.

REF **RE75021**

Σ **12x8**

2-8°C

EU: IVD CE

U.S.: *For research use only.*
Not for use in diagnostic procedures.

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1. INTENDED USE

Enzyme immunoassay for the qualitative and quantitative determination of IgM antibodies against cardiolipin in human serum.

2. SUMMARY AND EXPLANATION

The anti-phospholipid syndrome (APS) is an autoimmune disorder which can comprise clinical conditions as venous and arterial thrombosis, thrombocytopenia, myocardial infarction, recurrent spontaneous abortion and neurological complications. Cardiolipin (CL) is the most common, negatively charged, acid phospholipid and autoantibodies to cardiolipin are characteristic of APS. Anti-cardiolipin autoantibodies are also present in some patients with SLE and related diseases which is typical for a secondary anti-phopholipid syndrome.
Autoantibodies associated with APS are directed not only against CL and similar phospholipides but also against phospholipid/protein complexes. ß2-glycoprotein 1 (ß2-GP1; apolipoprotein H) has been identified as such a natural and essential co-antigen for CL-autoantibodies.

3. TEST PRINCIPLE

Solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The wells are coated with antigen. Specific antibodies of the sample binding to the antigen coated wells are detected by a secondary enzyme conjugated antibody (E-Ab) specific for human IgM. After the substrate reaction the intensity of the color developed is proportional to the amount of IgM-specific antibodies detected. Results of samples can be determined directly using the standard curve.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic use* only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Observe lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop Solution. It may cause skin irritations and burns.
9. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
10. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

The microtiter strips are stable up to the expiry date of the kit in the broken, but tightly closed bag when stored at 2-8°C, together with the desiccant.

6. SPECIMEN COLLECTION AND STORAGE

Serum

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	-20°C	Keep away from heat or direct sun light. Avoid repeated freeze-thaw cycles.
Stability:	7 d	> 7 d	

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Coated with cardiolipin and ß2-GP1.
1 x 14 mL	ENZCONJ IgM	**Enzyme Conjugate IgM** Ready to use, green colored. Contains: anti-human IgM, conjugated to peroxidase, protein-containing buffer and preservatives.
1 x 6 x 2 mL	CAL A–F	**Calibrator A–F** 0; 3.0; 8.0; 18; 45; 100 MPL-U/mL. Ready to use, gradually blue colored. Contains: IgM antibodies against cardiolipin, TBS and preservatives.
1 x 2 mL	CONTROL +	**Positive Control** Ready to use, red colored. Contains: IgM antibodies against cardiolipin, TBS and preservatives.
1 x 2 mL	CONTROL -	**Negative Control** Ready to use, green colored. Contains: TBS and preservatives.
1 x 100 mL	SAMPLEDIL	**Sample Diluent** Ready to use, orange colored. Contains: TBS and preservatives.
1 x 14 mL	TMB SUBS	**TMB Substrate Solution** Ready to use, colorless. Contains: TMB, hydrogen peroxide.
1 x 100 mL	WASHBUF CONC	**Wash Buffer,** Concentrate (10x) Blue colored. Contains: TBS and preservatives.
1 x 14 mL	STOP	**TMB Stop Solution** Ready to use, colorless. 0.5 M H_2SO_4.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3% CV). Volumes: 5; 100; 500 µL
2. Calibrated measures
3. Tubes (1 mL) for sample dilution
4. 8-Channel Micropipettor with reagent reservoirs
5. Wash bottle, automated or semi-automated microtiter plate washing system
6. Microtiter plate reader capable of reading absorbance at 450 nm (reference wavelength 600-650 nm)
7. Bidistilled or deionised water
8. Paper towels, pipette tips and timer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25°C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.

3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Use a pipetting scheme to verify an appropriate plate layout.
5. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
6. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
7. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of Components

Dilute / dissolve	Component		Diluent	Relation	Storage	Stability
100 mL	WASHBUF CONC	ad 1000 mL	bidist. water	1:10	2-8°C	4 weeks

10.2. Dilution of Samples

Sample	to be diluted	With	Relation	Remarks
Serum	generally	SAMPLEDIL	1:101	e.g. 5 µL + 500 µL

Samples containing concentrations higher than the highest Calibrator have to be diluted further.

11. TEST PROCEDURE

1.	Immediately prior to use, wash the solid phase once: fill wells with **300 µL Wash Buffer** each, soak for about 10 seconds in the wells and remove.
2.	Pipette **100 µL** of each **Calibrator, Control and diluted sample** into the respective wells of the Microtiter Plate. The reliability of the analysis can be improved by duplicate determinations.
3.	**Incubate 30 min** at **room temperature (18-25°C).**
4.	Discard incubation solution. Wash plate **3 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
5.	Pipette **100 µL** of **Enzyme Conjugate** into each well.
6.	**Incubate 30 min** at **room temperature (18-25°C).**
7.	Discard incubation solution. Wash plate **3 x** with **300 µL** of diluted **Wash Buffer.** Remove excess solution by tapping the inverted plate on a paper towel.
8.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
9.	Pipette **100 µL** of **TMB Substrate Solution** into each well.
10.	**Incubate 30 min** at **room temperature (18-25°C)** (protect from direct sunlight).
11.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate. Color changes from blue to yellow.
12.	**Measure** optical density with a photometer at **450 nm** (Reference-wavelength: 600-650 nm) within **30 min** after pipetting of the Stop Solution.

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. The Positive and Negative Control must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

It is recommended to participate at appropriate quality assessment trials.

13. CALCULATION OF RESULTS

The data obtained are **quantitatively** evaluated with a standard curve using a conventional ELISA evaluation program as shown in the example below. A good fit is provided with a 4-parameter function or spline approximation. Alternatively, the standard curve may also be drawn by hand on semi-logarithmic graph paper. The obtained absorbance values (ODs) of the Calibrators (y-axis, linear) are plotted against their concentration (x-axis, logarithmic).

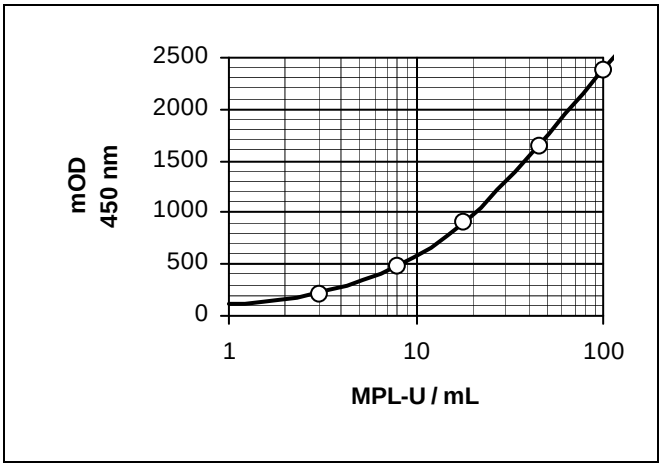
The concentration of the samples can be read directly from the standard curve. The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution have to be multiplied with the dilution factor.

Samples showing concentrations above the highest Calibrator have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

Typical Calibration Curve

(Example: Do not use for calculation!)

Calibrator	MPL-U/mL	Mittelwert OD
A	0	0.054
B	3.0	0.222
C	8.0	0.480
D	18	0.903
E	45	1.650
F	100	2.388



The test may also be evaluated in a **qualitative** manner. This requires measurement of only Positive Control. Nevertheless, measurement and examination of Negative Control is recommended (see: quality control).

In the qualitative test evaluation, the absorbance (OD) of the samples is compared with the borderline OD (= cut-off). The borderline OD is determined according to the following formula:

$$OD_{borderline} = OD_{positive\ control} \times factor$$

The factor depends on the kit lot and is quoted in the lot-specific QC Certificate which is included with each test kit. Example:

$OD_{positive\ control}$ = 1.270
factor = 0.37
$OD_{borderline}$ = 1.270 x 0.37 = 0.470

In order to gain an impression of the degree of a sample's reactivity, one may calculate the ratio, according to the formula below.

$$ratio = OD_{sample} / OD_{borderline}$$

Example:

$OD_{borderline}$ = 0.470
OD_{sample} = 1.425
ratio = 1.425 / 0.470 = 3.0

14. INTERPRETATION OF RESULTS

Interpretation	Quantitative evaluation MPL-U/mL	Qualitative evaluation Ratio
negative	< 4.5	< 0.85
cut-off	6.0	1.0
equivocal	4.5 - 7.5	0.85 - 1.15
positive	> 7.5	> 1.15

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

15. EXPECTED VALUES

In a sera collective of 160 blood donors, equally distributed by sex and age, the following distribution of the analyte was determined:

Mean: 2.5 MPL-U/mL
Mean + 2 SD: 8.0 MPL-U/mL
Median: 1.5 MPL-U/mL
95 % percentile: 4.5 MPL-U/mL

16. LIMITATIONS OF THE PROCEDURE

Specimen collection has a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details.

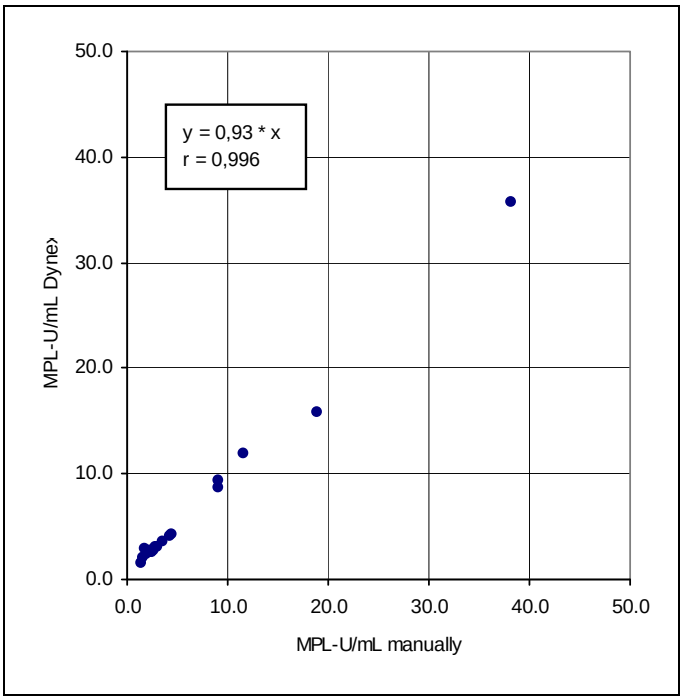
Azide and thimerosal at concentrations > 0.1 % interfere in this assay and may lead to false results.

17. PERFORMANCE

Standardisation	Calibrated against a set of commercially available, gradually positive sera ("Harris sera"; Louisville APL Diagnostics Inc., Louisville, KY, USA).			
Analytical Sensitivity	Detection limit (3 x SD of sample buffer) < 0.5 MPL-U/mL (n = 24)			
Analytical Specificity	Specific for human IgM against cardiolipin			
Precision	**Intra-assay variability (n = 24)**		**Inter-assay variability (n = 72)**	
	Mean (MPL-U/mL)	% CV	Mean (MPL-U/mL)	% CV
	8.0	2.7	9.7	3.3
	20	5.6	16	6.4
	43	3.2	52	3.8
	Operator to operator variability (n = 12)		**Variability between 2 kit lots (n = 6)**	
	Mean (MPL-U/mL)	% CV	Mean (MPL-U/mL)	% CV
	11	3.0	7.5	7.1
	17	6.2	21	6.4
	56	6.1	41	8.2
Linearity	1.5 - 82 MPL-U/mL			

18. AUTOMATION

Manually versus Dynex DS2



Variability: Using specimen of one and the same kit lot, the variability of assay results were compared between manual operation and the Dynex DS2 automated ELISA system:

	manual operation		Dynex DS2	
	Sample 1	Sample 2	Sample 1	Sample 2
Intra-Assay Variability (n = 8) CV_{Mean}	1.5 %	1.7 %	2.5 %	3.5 %
Inter-Assay Variability (n = 24) CV_{Mean}	2.0 %	1.8 %	3.6 %	4.8 %

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

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