Instructions for Use

Phospholipid-Ab IgG/IgM Screen ELISA

Enzyme immunoassay for the separate quantitative detection of IgG and/or IgM antibodies against phospholipids in human serum

REF **RE70411**

Σ **96**

EU: IVD CE

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

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Instruction manual

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1. Intended Use

Phospholipid-Ab IgG/IgM Screen ELISA is a solid phase enzyme immunoassay for the separate quantitative detection of IgG and/or IgM antibodies against phospholipids in human serum. Each well is coated with highly purified bovine cardiolipin + ß2-glycoprotein I, phosphatidyl- serine, -inositol, -ethanolamin, - choline and sphingomyelin .

The assay is an aid in the diagnosis and risk estimation of thrombosis in patients with systemic lupus erythematosus and antiphospholipid syndrome (APS).

2. Clinical Application and Principle of the Assay

Antibodies against phospholipids, components of the biological membranes, are specific for phospholipids such as Cardiolipin, Phosphatidyl -inositol, -ethanolamine, -choline, Sphingomyelin and phosphatidic acid.

Anti-phospholipid antibodies are frequently found in sera of patients with systemic lupus erythematosus (SLE) and related diseases. The occurrence of anti-phospholipid antibodies in patients with SLE and related diseases is typical for a secondary anti-phospholipid syndrome (APS). In contrast, anti-phospholipid antibodies in patients with no other autoimmune diseases characterize the primary APS.

Many studies have shown a correlation between these autoantibodies and an enhanced incidence of thrombosis, thrombocytopenia and habitual abortions (as a consequence of placental infarct). The exact mechanisms by which pathogenic anti-phospholipid antibodies induce thrombosis is not yet revealed fully.

Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

3. Kit Contents

To be reconstituted:

5x Sample Buffer 1 vial, 20 ml - 5x concentrated (capped white: yellow solution)
Containing: Tris, NaCl, BSA, sodium azide (preservative)

50x Wash Buffer 1 vial, 20 ml - 50x concentrated (capped white: green solution)
Containing: Tris, NaCl, Tween, sodium azide (preservative)

Ready to use:

Negative Control 1 vial, 1.5 ml (capped green: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)

Positive Control 1 vial, 1.5 ml (capped red: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)

Calibrators 6 vials, 1.5 ml each 0, 3, 10, 30, 100, 300 U/ml
(color increasing with concentration: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)

Conjugates 1 vial,15 ml IgG (capped blue: blue solution)
1 vial,15 ml IgM (capped green: green solution)
Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase

TMB Substrate 1 vial, 15 ml (capped black)
Containing: Stabilized TMB/H_2O_2

Stop Solution 1 vial, 15 ml (capped white: colorless solution)
Containing: 1M Hydrochloric Acid

Microtiterplate 12x 8 well strips with breakaway microwells
Coating see paragraph 1

Material required but not provided:

Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glass ware, test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or multipipette. Microplate washing device (multichannel pipette or automated system), adsorbent paper.
Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).

4. Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 4°C/39°F, at least. ***Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.***

5. Precautions of Use

5.1 Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety :

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.
Do not smoke, eat or drink when manipulating the kit.
Do not pipette by mouth.
All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases and according to national requirements.

5.2 General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-26°C/64-78.8°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Never expose components to higher temperature than 37°C/ 98,6 °F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated.

6. Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements.

Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes. After separation, the serum samples should be used immediately, respectively stored tightly closed at 2-8°C/35-46°F up to three days, or frozen at -20°C/-4°F for longer periods.

7. Assay Procedure

7.1 Preparations prior to pipetting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).
Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

Samples

Dilute serum samples 1:101 with sample buffer (1x)
e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well !

Washing

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells
e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

7.2 Work flow

NOTE: If IgG and IgM are determined in parallel, calibrators, controls and samples have to be done twice, for each subclass separately.

- Pipette 100 µl of each patient‘s diluted serum into the designated microwells.
- Pipette 100 µl calibrators and negative and positive controls into the designated wells.
- Incubate for 30 minutes at room temperature (20-26°C/64-78.8°F).
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl conjugate into each well.
- Incubate for 15 minutes at room temperature (20-26°C/64-78.8°F).
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl TMB substrate into each well.
- Incubate for 15 minutes at room temperature (20-26°C/64-78.8°F), in the dark.
- Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
- Incubate 5 minutes minimum.
- Agitate plate carefully for 5 sec.
- Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

8. Quantitative Interpretation

For the **quantitative interpretation** establish the standard curve by plotting the ***optical density (OD) of each calibrator (y-axis)*** with respect to the corresponding concentration values in ***U/ml (x-axis)***. For best results we recommend log/lin coordinats and 4-Parameter Fit. From the OD of each sample, read the corres-ponding antibody concentrations expressed in ***U/ml***.

Normal Range	**Positive Results**
≤ 15 U/ml	**> 15 U/ml**

Example of a standard curve

We recommend pipetting calibrators in parallel for each run.

Calibrators IgG	***OD 450/620 nm***	***CV %***
0 U/ml	0.056	2.5
3 U/ml	0.144	1.5
10 U/ml	0.311	2.4
30 U/ml	0.623	3.2
100 U/ml	1.228	3.1
300 U/ml	2.091	0.9

Example of calculation

Patient	**Replicate (OD)**	**Mean (OD)**	**Result (U/ml)**
P 01	1.357/1.334	1.346	116.2
P 02	0.790/0.781	0.785	45.7

Do not use this example for interpreting patients results!

We recommend to retest samples, that are borderline. For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations.

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

9. Technical Data

Sample material:	serum
Sample volume:	10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time:	60 minutes at room temperature (20-26°C/64-78,8°F)
Calibration range:	0-300 U/ml
Analytical sensitivity:	1.0 U/ml
Storage:	at 2-8°C use original vials, only
Number of determinations:	96 tests

10. Performance Data

10.1 Analytical sensitivity

The analytical sensitivity of this kit has been found at 1.0 U/ml.

10.2 Specificity and sensitivity

The microplate is coated with β-2-glycoprotein I, cardiolipin, phophatidylcholin, -ethanolamin, -inostol, -serine and sphingomyelin. No crossreactivities to other autoantigens have been found.
The diagnostic sensitivity for the antigen β-2GPI is 47% and for cardiolipin it is 100%.

10.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

Sample No.	Dilution Factor	measured concentration	expected concentration	Recovery (%)
1	1 / 100	226.0	224.0	100.9
	1 / 200	109.0	112.0	97.3
	1 / 400	57.0	56.0	101.8
	1 / 800	26.0	28.0	92.9
2	1 / 100	170.0	173.0	98.7
	1 / 200	87.0	86.5	100.6
	1 / 400	41.5	43.3	95.8
	1 / 800	20.8	21.6	96.3

10.4 Precision

To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

Intra-Assay			Inter-Assay		
Sample No.	Mean (U/ml)	CV (%)	Sample No.	Mean (U/ml)	CV (%)
1	228.0	6.3	1	217.0	5.8
2	169.0	4.8	2	154.0	2.8
3	59.0	3.2	3	53.0	1.6

10.5 Calibration

Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml).

11. Literature

1. **Boey, M.L., Colaco, C.B., Gharavi, A.E., et al. (1983):**
   Thrombosis in systemic lupus erythematosus: striking association with the presence of circulating lupus anticoagulant.
   Br. Med. J. 287: 1021-1023.

2. **Gastineau, D.A., Kazmier, F.J., Nichols, W.L., Bowie, E.J. (1985):**
   Lupus anticoagulant: an analysis of the clinical and laboratory features of 219 cases.
   Am. J. Hematol. 19: 265-267.

3. **McNeil HP, Simpson RJ, Chesterman CN, Kirilis SA (1990):**
   Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: ß2-Glycoprotein I (apolipoprotein H).
   Proc Natl Acad Sci USA 87: 4120-4124.

4. **Wöhrle R, Matthias T, von Landenberg P, Oppermann M, Helmke K, Förger F (2000):**
   Clinical relevance of antibodies against different phospholipids.
   Journal of Autoimmunity 15: A60.

Pipetting Scheme

		Calibrators (A-F)	Controls	Samples
Pipette **Pipette** **Pipette**	Calibrators (A-F) Controls Prediluted samples (1:101)	**100 µl each**	**100 µl each**	**100 µl each**
Incubate		***30 min at room temperature (20-26°C/64-78.8°F)***		
Decant		***Wash 3x with 300 µl of wash buffer (1x)***		
Pipette	Conjugate	**100 µl**	**100 µl**	**100 µl**
Incubate		***15 min at room temperature (20-26°C/64-78.8°F)***		
Decant		***Wash 3x with 300 µl of wash buffer (1x)***		
Pipette	Substrate	**100 µl**	**100 µl**	**100 µl**
Incubate		***15 min at room temperature (20-26°C/64-78.8°F), in the dark.***		
Pipette	Stop Solution	**100 µl**	**100 µl**	**100 µl**
Incubate		***5 min at room temperature (20-26°C/64-78.8°F)***		

Agitate plate for 5 seconds and read OD at λ450 nm (optionally λ450/620 nm) within 30 minutes. Resulting color is stable for 30 minutes, at least.

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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