Anti-Phospholipid-8 IgG/IgM EIA

For the separate qualitative detection of IgG and/or IgM antibodies against phospholipids in human serum.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 35-P8PHU-E01
Size: 96 wells
Version: 004: 2016-08-10 – ALPCO 1.0

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1. Intended Use

Anti-Phospholipid-8 IgG/IgM EIA is a solid phase enzyme immunoassay for the separate qualitative detection of IgG and/or IgM antibodies against phospholipids in human serum. The assay employs highly purified human ß2-Glycoprotein I, Cardiolipin + ß2-Glycoprotein I, Cardiolipin and Phosphatidylcholine, -ethanolamine, -inositol, -serine, and Sphingomyelin. The assay is for research use only. Not for use in diagnostic procedures.

2. Principle of the Assay

Serum samples diluted 1:101 are incubated in microplates coated with the specific antigen. Antibodies, if present in the sample, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards antihuman 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 intensity of color formed from the reaction is a function of the amount of conjugate bound to the antigen-antibody complex and is proportional to the initial concentration of the respective antibodies in the sample.

3. Kit Contents

To Be Reconstituted				
Item	**Quantity**	**Cap Color**	**Solution color**	**Description/Contents**
Sample Buffer (5x)	1 X 20 mL	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 X 20 mL	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
Ready to Use				
Item	**Quantity**	**Cap color**	**Solution color**	**Description / Contents**
Negative Control	2 x 1.8 mL	Green	Colorless	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Cut-off Calibrators	8 x 1.5 mL	White	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1 % (preservative)
Conjugate IgG IgM	1 x 15 mL 1 x 15 mL	Blue Green	Blue Green	Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15 mL	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)
Stop Solution	1 x 15 mL	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	**N/A**	**N/A**	With breakaway microwells. See first paragraph for description.

Materials Required, but Not Provided
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glassware (cylinder 100 - 1000mL), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µL) or adjustable multipipette (100-1000µL). Microplate washing device (300 µL repeating or 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 at 2-8°C/35-46°F for 1 month. ***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 research use only.

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin, it is recommended to avoid contact with eyes and skin and wear disposable gloves.

WARNGING! Standards, Controls, and Buffers contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or absorbed by skin or eyes. NaN_3 may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Refer to decontamination procedures outlined by the CDC or regulatory guidelines.

Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

All human source material used in kit reagents (e.g., controls, standards) 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 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 (20-32°C/68-89.6°F) before use, mix well, and follow the recommended incubation scheme for an optimum performance of the test.

Incubation: It is recommended to test performance at 30°C/86°F for automated systems.

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 previously for other reagents.

6. Sample Collection, Handling and Storage

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

Do not use icteric, lipemic, hemolyzed, 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 during the first 8 hours, stored tightly closed at 2-8°C/35-46°F up to two days (48 hours), 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).
To avoid mistakes, it is suggested to mark the cap of the different standards.

Samples
Dilute serum samples 1:101 with 1x sample buffer: 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/3546°F).

7.2 Recommended Pipetting Scheme

Antigen		1	2	3	4…	
β-2 Glyco. I	A	CCA	NC	S1	S2	S3
β2 Cardiolipin	B	CCB	NC	S1	S2	S3
Cardiolipin	C	CCC	NC	S1	S2	S3
P. – cholin	D	CCD	NC	S1	S2	S3
P. - ethanolamine	E	CCE	NC	S1	S2	S3
P. - inositol	F	CCF	NC	S1	S2	S3
P. - serine	G	CCG	NC	S1	S2	S3
Sphingomyelin	H	CCH	NC	S1	S2	S3

NC: negative control
CCA – CCH: cut-off calibrator
S1: sample 1
S2: sample 2
S3: sample 3

7.3 Test Steps

It is recommended to pipette samples and standards in duplicate.

1.	Ensure preparations from step 7.1 above have been carried out prior to pipetting.
2.	Pipette 100 µl of cut-off calibrator, negative control, and diluted serum samples into the designated microwells.
3.	Incubate for 30 minutes at 20-32°C/68-89.6°F.
4.	Wash 3x with 300 µl washing buffer (diluted 1:50).
5.	Pipette 100 µl conjugate into each well.
6.	Incubate for 30 minutes at 20-32°C/68-89.6°F.
7.	Wash 3x with 300 µl washing buffer (diluted 1:50).
8.	Pipette 100 µl TMB substrate into each well.
9.	Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
10.	Pipette 100 µl of stop solution into each well, using the same order as when pipetting the substrate.
11.	Incubate 5 minutes, minimum.
12.	Agitate plate carefully for 5 sec.
13.	Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

8. Qualitative Interpretation

Read the optical density of the specific standards (A-H) and samples. Multiply the OD of the standard by the parameter specific factor, provided with the lot specific QC certificate.

Phosholipid-8Pro	**O.D. 450/620 nm**
Low Control	0.033
Standard	0.5225

This kit is for research use only! Do not use in diagnostic procedures!

For lot specific data, see enclosed quality control leaflet. Laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU or other regulations and/or internal pooled sera, as foreseen by EU or other regulations.

For semi-quantification of the results, each sample-OD value can be expressed by the IndexValue. The Index-Value is calculated by dividing the sample-OD by the cut-off parameter:

$$\text{Index Value} = \frac{\text{OD (sample)}}{\text{OD (Cut-off parameter)}}$$

In case values of the controls do not meet the criteria the test is invalid and should be repeated. The following technical issues should be verified: expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions, and washing methods.

9. Technical Data

Sample material: Serum
Sample volume: 10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time: 90 minutes at 20-32°C/68-89.6°F)
Storage: at 2-8°C/35-46°F use original vials, only
Number of determinations: 96 tests

10. Performance Data

10.1 Specificity and sensitivity

The microplate is coated with ß2-Glycoprotein I, Cardiolipin + ß2-Glycoprotein I, Cardiolipin, Phophatidylcholine, -ethanolamine, -inostol, -serine and Sphingomyelin. No crossreactivities to other autoantigens have been found. Since Phospholipid-8 Pro consists of various antigens, the known values for IgG sensitivity and specificity are listed in the table below:

	Sensitivity	Specificity
Cardiolipin	67%	73%
ß2Glyco I	69%	69%
Phosphatidylserine	62%	83%
Phosphatidyl-Inositol	69%	75%
Ethanolamine	62%	78%
Choline	62%	79%

10.2 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 (OD-Ratio)	Expected Concentration (OD-Ratio)	Recovery (%)
1	1/ 100	7.40	7.50	98.7
	1/ 200	3.50	3.75	93.3
	1/ 400	1.75	1.88	93.1
	1/ 800	0.88	0.94	93.6
2	1/ 100	3.60	3.50	102.9
	1/ 200	1.71	1.75	97.7
	1/ 400	0.85	0.88	96.6
	1/ 800	0.43	0.44	97.7

10.3 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		
Phospholipid-8Pro-GM	**Mean OD-Ratio**	**CV (%)**
ß2-Glykoprotein	2.4	3.1
Cardiolipin + ß2-GP I	1.8	2.8
Cardiolipin	1.5	1.5
Phosphatidyl-Choline	3.2	1.6
Phosphatidyl-Ethanolamine	3.5	1.9
Phosphatidyl-Inosito	3.1	2.2
Phosphatidyl-Serine	2.6	2.9
Sphingomyelin	2.1	3.1

Inter-Assay		
Phospholipid-8Pro-GM	**Mean OD-Ratio**	**CV (%)**
ß2-Glykoprotein	2.8	2.8
Cardiolipin + ß2-GP I	1.5	3.1
Cardiolipin	1.3	3.6
Phosphatidyl-Choline	2.6	2.1
Phosphatidyl-Ethanolamine	2.9	3.5
Phosphatidyl-Inosito	4.1	2.6
Phosphatidyl-Serine	3.8	1.5
Sphingomyelin	3.6	2.4

11. Literature

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