Anti-Phosphatidylserine ELISA

Catalog Number:
PDL31-K01

Enzyme immunoassay for the determination of IgG and / or IgM antibodies to phosphatidyl-serine in human serum

20A Northwest Blvd., Suite 112,
Nashua, NH 03060
Phone: 617-419-2019
Fax: 617-419-1110
www.EagleBio.com

For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.
v. 1.0

INTENDED USE

The Eagle Biosciences Anti-Phosphatidylserine ELISA Assay Kit is used for the separate quantitative determination of IgG and / or IgM antibodies to phosphatidylserine in human serum for the diagnosis of anti-phospholipid antibody syndrome (APAS).

APAS is an autoimmune disorder comprising such clinical symptoms like arterial or venous thrombosis, thrombocytopenia and recurrent fetal loss. Primary APAS as well as systemic lupus erythematosus (SLE) are characterized by the appearance of autoantibodies to negatively charged phospholipids (1). Although significance and pathological relevance of phospholipid antibodies are not completely revealed yet, the detection of such autoantibodies is widely established and plays an important role in the diagnostics of systemic autoimmune diseases.

Unlike phospholipid antibodies which appear in some infectious disease patients autoimmune patients exhibit phospholipid antibodies that seem to recognize phospholipids in association with plasma protein cofactors such as ß2-glycoprotein I (ß2-GP I, apolipoprotein H) (2). ß2-GP I, a serum protein with a molecular weight of 50 kDa, affects platelet aggregation and coagulation.

The positively charged fifth domain of ß2-GP I interacts with negatively charged phospholipids such as phosphatidyl-serine. This interaction results in conformational changes of the protein and the creation of new epitopes apparently recognized by autoimmune phospholipid autoantibodies.

(1) Harris EN, Gharavi AE, Boey ML, Patel BM, Mackworth-Young GG, Loizou S and Hughes GRV: Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. Lancet 1983 11:1211
(2) McNeil HP, Simpson RJ, Chesterman CN, Krilis SA: Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding factor of coagulation: beta 2-glycoprotein I (apolipoprotein H). Proc Natl Acad Sci USA 1990 87:4120-4124

PRINCIPLE OF THE TEST

The Eagle Biosciences Anti-Phosphatidylserine ELISA Assay Kit is used for the highly sensitive determination of autoantibodies to phosphatidyl-serine in human serum.

The antibodies of calibrators, control and diluted patient samples react with an antigen complex consisting of phosphatidyl-serine and the cofactor ß2-GP I immobilized on the solid phase of microtiter plates. Following an incubation period of 60 min at room temperature, unbound sample components are removed by a wash step.

The bound antibodies react specifically with anti-human-IgG or anti-human-IgM conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at room temperature (RT). Excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the antibody concentrations of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve. Evaluating the test by a semi-quantitative method using a cut-off calibrator is also possible.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, hemolytic or contaminated samples should not be run. The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at – 20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: ***Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1.0 ml sample diluent (C), prior to assay.***

TEST COMPONENTS FOR 96 DETERMINATIONS

A Ag 96	**Microtiter plate**, 12 breakable strips per 8 wells with phosphatidyl-serine (bovine) and ß2-GP I (human)	1 vacuum sealed with desiccant
B BUF WASH	**Concentrated wash buffer** sufficient for 1000 ml solution 10x	100 ml concentrate capped white
C DIL	**Sample diluent**	100 ml ready for use capped black
D CONJ G	**Conjugate IgG** containing anti-human-IgG- (sheep) coupled with horseradish peroxidase	15 ml ready for use capped red
E CONJ M	**Conjugate IgM** containing anti-human-IgM- (sheep) coupled with HRP	15 ml ready for use capped green
F SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
G H2SO4	**Stop solution** 0.25 sulfuric acid 0.25 M	15 ml ready for use capped yellow
0 - 4 CAL	**Calibrators** (diluted sera) conc.: 1, 10, 30, 100, 300 U/ml	1 ml each ready for use capped white
P CONTROL	**Positive control** (diluted serum) conc.: see leaflet enclosed +	1 ml ready for use capped red
N CONTROL	**Negative control** (diluted serum) conc.: see leaflet enclosed -	1 ml ready for use capped green

Materials required in addition

- Adjustable micropipettes 10 - 100 µl, 100 – 1000 µl
- pipette tips
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- distilled or de-ionized water

Size and storage

Anti-Phosphatidylserine has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the Anti-Phosphatidylserine have to be kept at 2…8°C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water.
For example, dilute 8 ml of the concentrate with 72 ml of distilled water. The wash solution prepared is stable up to 30 days at 2…8 °C.

All other test components are ready for use and stable up to the expiration date printed on each vial.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- **Dilute patient sera with sample diluent (C) 1+100 (v/v), e.g. 10 µl serum + 1.0 ml sample diluent (C)**.
- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature (20…25°C) before use. Mix gently without causing foam.
2. Dispense
   100 µl calibrators (0 optional) 1 - 4 (quantitative) or
   100 µl calibrator 1 (semi-quantitative)
   100 µl Controls P, N (N optionally)
   100 µl diluted patient samples
   into the respective wells.
3. Incubate **60 min** at room temperature (20…25°C).
4. Decant, then wash each well **three** times using **300 µl**
   wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Incubate **30 min** at room temperature (20…25°C).
7. Decant, then wash each well **three** times using **300 µl**
   wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **15 min protected from light** at room temperature (20…25°C).
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within 30 min after adding the stop solution.

DATA PROCESSING

Anti-Phosphatidylserine allows both the quantitative (4 + 1 calibrators) and semi-quantitative (calibrator 1 for cut-off determination) evaluation of the results.

Quantitative evaluation

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 4 (CAL 0 optionally) on the ordinate, y-axis, (lin. scale) versus their respective anti-phosphatidylserine concentrations on the abscissa, x-axis, (log. scale). Anti-phosphatidylserine concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary,

as all other components of the kit are supplied accordingly.

Semi-quantitative evaluation

Results are interpreted by calculating the binding index (BI) using **calibrator 1 (10 U/ml)** as **cut-off calibrator**. The BI is the ratio of the OD-value of a sample to the cut-off OD-value (CAL 1).

BI = OD_{sample} / ($OD_{calibrator\ 1}$)

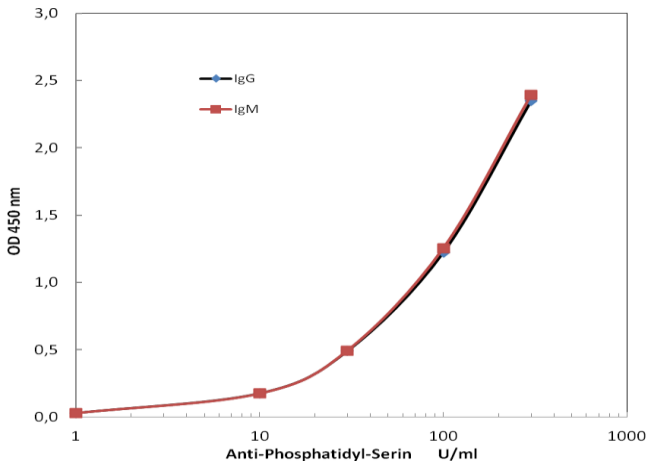
Both evaluation variants of Anti-Phosphatidyl-Serin may be achieved also with computer assisted analysis software intergrated in the photometers.

Example of Typical Assay Results

IgG	OD 1	OD 2	MW OD	U/ml
Calibrator **0**	0.027	0.030	0.029	**1**
Calibrator **1**	0.175	0.176	0.176	**10**
Calibrator **2**	0.468	0.512	0.490	**30**
Calibrator **3**	1.154	1.293	1.224	**100**
Calibrator **4**	2.297	2.402	2.350	**300**
Patient **1**	1.082	1.146	1.114	**87**

IgM	OD 1	OD 2	MW OD	U/ml
Calibrator **0**	0.029	0.028	0.029	**1**
Calibrator **1**	0.172	0.176	0.174	**10**
Calibrator **2**	0.493	0.494	0.494	**30**
Calibrator **3**	1..243	1.267	1.255	**100**
Calibrator **4**	2.401	2.381	2.391	**300**
Patient **1**	0.511	0.535	0.523	**33**

TYPICAL STANDARD CURVES



Specimens with an OD > calibrator 4 should be diluted with higher volumes of sample diluent and tested again. Results are multiplied with the dilution factor chosen.

Test validity

The test run is valid if:

- the mean OD of the calibrator 4 is ≥ 1.2
- Concentration of Control P see leaflet enclosed
- Control N is negative

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

REFERENCE VALUES

Anti-Posphatidyl-Serin IgG and IgM	U/ml	BI
positive	**≥ 10**	**≥ 1,0**
negative	**< 10**	**< 1,0**

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum Anti-Phosphatidylserine levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method

Healthy individuals should be tested negative by the Anti-Phosphatidylserine. However, Anti-Phosphatidylserine autoantibody positive apparently healthy persons do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

CHARACTERISTIC ASSAY DATA

Calibration

No international reference material for this parameter is available, there for so the assay is calibrated in arbitrary units.

Linearity

Selected positive serum samples have been tested by this assay and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be sera that do not follow this rule.

Specificity

Measuring a group of blood donors (n=62) in both Anti-Phosphatidyl-Serin IgG and IgM assays a specificity of 100% was found. All samples were tested negative.

Precision

Intra-Assay coefficient of variation (CV): 20-fold determination

	IgG		IgM	
sample	**U/ml**	**CV (%)**	**U/ml**	**CV (%)**
1	158.7	8.9	96.7	7.6
2	37.0	9.9	14.5	7.3
3	5.8	9.6	3.2	3.7

Inter-Assay cefficient of variation (CV): 5 different runs of 10-fold determinations:

	IgG		IgM	
sample	**U/ml**	**CV (%)**	**U/ml**	**CV (%)**
1	152.7	13.4	108.2	11.4
2	36.2	9.9	15.2	6.4
3	5.4	13.2	3.3	3.6

ASSAY SCHEME

Dilute patients sample 10 µl serum + 1.0 ml sample diluent (C)

1	Bring all ready for use reagents to room temperature (20…25°C) before use.			
		calibrators	controls	sera
2	Pipette calibrators (0 - 4) or calibrator 1	100 µl		
	controls (P, N)		100 µl	
	1 + 100 prediluted patient sera			100 µl
3	Incubate	60 minutes at room temperature (20…25°C)		
4	Wash	Decant, 3 x 300 µl (made of B)		
5	Pipette conjugate (D)	100 µl	100 µl	100 µl
6	Incubate	30 minutes at room temperature (20…25°C)		
7	Wash	Decant, 3 x 300 µl (made of B)		
8	Pipette substrate (E)	100 µl	100 µl	100 µl
9	Incubate protected from light	15 minutes at room temperature (20…25°C)		
10	Pipette stop solution (F)	100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm within 30 min.			

SAFETY PRECAUTIONS

- **This Anti-Phosphatidylserine ELISA Assay Kit is for research use only**. Follow the working instructions carefully. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2…8°C before use in the original shipping container.
- Some of the reagents contain small amounts of Neolone™ M10 (≤ 1.0% v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the Anti-Phosphatidylserine kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.