DRG® Anti-Phosphatidyl Serine IgG / IgM (EIA-3592)

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This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Please use only the valid version of the package insert provided with the kit.

NAME AND INTENDED USE

Anti-Phosphatidyl Serine is an indirect solid phase enzyme immunoassay (ELISA) for measurement of IgG and IgM class autoantibodies against phosphatidyl serine in human serum or plasma.

PRINCIPLE OF THE TEST

Highly purified phosphatidyl serine is bound to microwells saturated with β2-glycoprotein I. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG and IgM immunologically detect the bound specimen sample antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG resp. IgM antibodies present in the original sample.

WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for research use only.
2. Do not interchange kit components from different lots.
3. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
4. Avoid contact with the TMB (3,3´,5,5´-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
5. Avoid contact with the Stop Solution which is acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN_3) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.).
7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.

9. Do not pipette by mouth.
10. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

CONTENTS OF THE KIT

Package size 96 determ.

Qty.1	Divisible **microplate** consisting of 12 modules of 8 wells each, coated with highly purified Phosphatidyl serine and saturated with β2-Glycoprotein I. Ready to use.
6 vials, 1.5 ml each	combined **Calibrators** with IgG and IgM class Anti-Phospholipid antibodies (A-F) in a serum/buffer matrix (PBS, BSA, NaN_3 <0,1% (w/w)) containing: **IgG**: 0; 6.3; 12.5; 25; 50; and 100 GPL U/ml and **IgM**: 0; 6.3; 12.5; 25; 50; 100 MPL U/ml. Ready to use.
2 vials, 1,5 ml each	Anti-Phospholipid **Controls** in a serum/buffer matrix (PBS, BSA,NaN_3 <0,1% (w/w)) positive (1) and negative (2), for the respective concentrations see the enclosed QC Certificate. Ready to use.
1 vial, 20 ml	**Sample buffer** (Tris, NaN_3 <0.1% (w/w)), yellow, concentrate (5x).
1 vial, 15 ml	**Enzyme conjugate** solution (PBS, PROCLIN 300 <0.5% (v/v)), (light red) containing polyclonal rabbit **anti-human IgG**; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	**Enzyme conjugate** solution (PBS, PROCLIN 300 <0.5% (v/v)), (light red) containing polyclonal rabbit **anti-human IgM**; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	**TMB substrate solution**. Ready to use.
1 vial, 15 ml	**Stop solution** (contains acid). Ready to use.
1 vial, 20 ml	**Wash solution** (PBS, NaN_3 <0.1% (w/w)), concentrate (50x).

STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8 °C.

MATERIALS REQUIRED

Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl

– Laboratory timing device
– Data reduction software

Preparation of reagents
– Distilled or deionized water
– Graduated cylinder for 100 and 1000 ml
– Plastic container for storage of the wash solution

SPECIMEN COLLECTION, STORAGE AND HANDLING

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8 °C for up to five days or stored at -20 °C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28 °C).
4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contamination change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the specimen sample results semi quantitatively.

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PREPARATION OF REAGENTS

Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all specimen samples **1:100** with sample buffer before assay.
Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well.

Controls are ready to use and need not be diluted.

TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted specimen samples.
2. Pipet 100 µl of calibrators, controls and prediluted specimen samples in duplicate into the wells.

	1	2	3	4	5	6
A	SA	SE	P1	P5		
B	SA	SE	P1	P5		
C	SB	SF	P2	P..		
D	SB	SF	P2	P..		
E	SC	C1	P3			
F	SC	C1	P3			
G	SD	C2	P4			
H	SD	C2	P4			

SA - SF: standards A to F
P1, P2... specimen sample 1, 2 ...
C1: positive control
C2: negative control

3. Incubate for 30 minutes at room temperature (20-28 °C).
4. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
5. Dispense 100 µl of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
8. Dispense 100 µl of TMB substrate solution into each well.

9. Incubate for 15 minutes at room temperature.
10. Add 100 µl of stop solution to each well of the modules and incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Automation

The Anti-Phosphatidyl Serine IgG/IgM ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

Calculation of results

For Anti-Phosphatidyl Serine IgG and IgM a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Calculation example

The figures below show typical results for anti-Phospholipid IgG and IgM. These data are intended for illustration only and should not be used to calculate results from another run.

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Calibrators										
	No	Position	OD 1	OD 2	Mean	Conc. 1	Conc. 2	Mean	decl. Conc.	CV %
IgG	STA	A 1/B 1	0.085	0.091	0.088	0.0	0.3	0.2	0.0	5
IgG	STB	C 1/D 1	0.277	0.222	0.225	6.0	5.8	5.9	6.3	2
IgG	STC	E 1/F 1	0.370	0.376	0.373	12.0	12.3	12.2	12.5	1
IgG	STD	G 1/H 1	0.687	0.703	0.695	26	27	27	25	2
IgG	STE	A 2/B 2	1.109	1.113	1.111	48	48	48	50	0
IgG	STF	C 2/D 2	1.911	1.881	1.896	102	100	101	100	1
IgM	STA	A 7/B 7	0.031	0.033	0.032	0.0	0.1	0.0	0.0	4
IgM	STB	C 7/D 7	0.239	0.249	0.244	6.1	6.3	6.2	6.3	3
IgM	STC	E 7/F 7	0.458	0.465	0.462	12.5	12.7	12.6	12.5	1
IgM	STD	G 7/H 7	0.791	0.826	0.809	24	26	25	25	3
IgM	STE	A 8/B 8	1.289	1.299	1.294	50	51	50	50	1
IgM	STF	C 8/D 8	1.791	1.784	1.788	101	99	100	100	0

REFERENCES

1. Falcon, C.R., A.M.Hoffer and L.O.Carreras. Antiphosphatidylinositol antibodies as markers of the antiphospholipid syndrome. Thromb. Haemost.Vol. 63, 321-322. 1990.
2. López-Soto, A., R. Cervera, J. Font et al. Isotype distribution and clinical significance of antibodies to cardiolipin, phosphatidic acid, phosphatidylinositol and phosphatidylserine in systemic lupus erythematosus: prospective analysis of a series of 92 patients. Clin. Exp. Immunol.Vol. 15, 143-149. 1997.
3. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RHWM, de Groot PG, Koike T, Meroni PL, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG, Krilis SA. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006; 4: 295-306.
4. Maneta-Peyret, L., C. Previsani,Y. Sultan et al. Autoantibodies against all the phospholipids: a comparative study with systemic lupus erythematosus and healthy sera.Eur. J.Clin.Chem. Biochem., Vol. 29, 39-43. 1991.
5. Toschi,V., A.Motta, C. Castelli et al. Prevalence and clinical significance of antiphospholipid antibodies to noncardiolipin antigens in systemic lupus erythematosus. Haemostasis, Vol. 23, 275-283. 1993.
6. Rauch, J., and A.S. Janoff. Antibodies against phospholipids other than cardiolipin: potential role for both phospholipid and protein. Lupus, Vol. 5, 498-502. 1996.
7. Weidmann, C.E., D.J.Wallace, J.B. Peter et al. Studies of IgG, IgM and IgA antiphospholipid antibody isotypes in systemic lupus erythematosus. J. Rheumatol., Vol. 15, 74-79. 1988.

8. Yodfat,O., M. Blank, I. Krause and Y. Shoenfeld.The pathogenic role of antiphosphatidylserine antibodies: active immunization with antibodies leads to the induction of antiphospholipid syndrome. Clin. Immunol. Immunopathol., Vol. 78, 14-20. 1996.