ELISA kits available from ADI (see details at the web site)

Catalog#	ProdDescription
3100	Human anti-dsDNA IgG ELISA Kit, 96 tests, Quantitative
3105	Human anti-dsDNA IgM ELISA Kit, 96 tests, Quantitative
3110	Human anti-dsDNA IgA ELISA Kit, 96 tests, Quantitative
3115	Human anti-ssDNA IgG ELISA Kit, 96 tests, Quantitative
3205	Human Anti-Nuclear Antibodies (ANA) ELISA Kit, 96 tests, Semi-Quantitative
3210-SSA	Human anti-SS-A (60 Kda/Ro IgG ELISA Kit, 96 tests, Quantitative
3215-SSA	Human anti-SS-A (52 Kda/Ro IgG ELISA Kit, 96 tests, Quantitative
3220-SSB	Human anti-SS-B/La IgG ELISA Kit, 96 tests, Quantitative
3250	Anti-thyroid peroxidase ELISA kit, Semi-Quantitative
3300	Anti-helicobacter pylori IgG ELISA kit, Semi-Quantitative
3300-100-SMG	Human Anti-Smith antigen (Sm) IgG ELISA kit, 96 tests, Quantitative
3300-110-SRG	Human Anti-Smith antigen/RNP (Sm/RNP) IgG ELISA kit, 96 tests,
3300-120-RNG	Human Anti-RNP (RNP-70) IgG ELISA kit, 96 tests, Quantitative
3300-130-HNG	Human Anti-histones IgG ELISA kit, 96 tests, Quantitative
3300-140-SCG	Human Anti-Scl-70 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG ELISA kit,
3300-150-JOG	Human Anti-Jo-1 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG ELISA kit, 96
3300-160-AFG	Human Anti-Alpha Fodrin IgG ELISA kit, 96 tests, Quantitative
3300-170-CLG	Human Anti-Cardiolipin IgG ELISA kit, 96 tests, Quantitative
3300-175-CLM	Human Anti-Cardiolipin IgM ELISA kit, 96 tests, Quantitative
3300-185-CLA	Human Anti-Cardiolipin IgA ELISA kit, 96 tests, Quantitative
3300-190-B2G	Human Anti-Beta2-Glycoprotein 1 IgG ELISA kit, 96 tests, Quantitative
3300-195-B2M	Human Anti-Beta2-Glycoprotein 1 IgM ELISA kit, 96 tests, Quantitative
3300-200-B2A	Human Anti-Beta2-Glycoprotein 1 IgA ELISA kit, 96 tests, Quantitative
3300-205-APS	Human Anti-Phospholipid Screen (anti-Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic Acid and beta-2-Glycoprotein I) IgG/IgM ELISA kit, 96 tests, Quantitative
3300-210-PSS	Human Anti-Phosphotidyl serine IgG/IgM ELISA kit, 96 tests, Quantitative
3300-215-PIS	Human Anti-Phosphotidyl Inositol IgG/IgM ELISA kit, 96 tests, Quantitative
3300-220-PAS	Human Anti-Phosphotidic Acid IgG/IgM ELISA kit, 96 tests, Quantitative
3300-230-APG	Human Anti-Prothrombin IgG/IgM ELISA kit, 96 tests, Quantitative
3300-235-APA	Human Anti-Prothrombin IgA ELISA kit, 96 tests, Quantitative
3300-240-AVA	Human Anti-Annexin V IgG ELISA kit, 96 tests, Quantitative
3300-250-ANG	Human ANCA Screen (Anti-PR3 and Anti-MPO) IgG ELISA kit, 96 tests,
3300-255-PRG	Human ANCA (Anti-PR3) IgG ELISA kit, 96 tests, Quantitative
3300-260-LFG	Human Anti-Lactoferrin IgG ELISA kit, 96 tests, Quantitative
3300-265-MPG	Human ANCA (Anti-MPO) IgG ELISA kit, 96 tests, Quantitative
3300-270-GBG	Human Anti-glomerular basement membrane (GBM) IgG ELISA kit, 96 tests,
3300-280-BPG	Human Anti-bactericidal permeability increasing (BPI) protein IgG ELISA kit, 96
3300-290-ELG	Human Anti-Elastase IgG ELISA kit, 96 tests, Quantitative
3300-300-GLG	Human Anti-Gliadin IgG ELISA kit, 96 tests, Quantitative
3300-305-GLM	Human Anti-Gliadin IgM ELISA kit, 96 tests, Quantitative
3300-310-GLA	Human Anti-Gliadin IgA ELISA kit, 96 tests, Quantitative
3300-315-PRG	Human Anti-Parietal cell (alpha and beta subunits of the Parietal Cell (H//K/ATPase) IgG ELISA kit, 96 tests, Quantitative
3300-320-ASC	Human Anti-ASCA (mannan from Saccharomyces cerevisiae) IgA/IgG ELISA kit, 96 tests, Quantitative
3300-330-ASG	Human Anti-Sperm IgG ELISA kit, 96 tests, Quantitative
3300-340-CCG	Human Anti-Cyclic Citrullinated Peptide (CCP) IgG ELISA kit, 96 tests,
3300-350-TPG	Human Anti-thyroid peroxidase (TPO) IgG ELISA kit, 96 tests, Quantitative
3300-360-TGG	Human Anti-thyroglobulin (TG) IgG ELISA kit, 96 tests, Quantitative

Instruction Manual No. M-3300-210-PSS

Anti-Phosphatidyl Serine IgG/IgM

ELISA KIT Cat. No. 3300-210-PSS

for the determination of IgG and IgM class Autoantibodies against Phosphatidyl Serine

For In Vitro Research Use Only

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Anti- Phosphatidyl Serine ELISA KIT Cat. No. 3300-210-PSS

Kit Contents: (reagents for 96 tests)

Components	
Highly purified phosphatidyl serine and saturated with beta-2-glycoprotein I coated microwell strip plate (96 wells);#3300211	96 wells (1 plate)
Std. A, 1.5 ml, 0.0 GPL/MPL-U/mL, #3300212A	1 vial
Std. B, 1.5 ml, 6.3 GPL/MPL-U/mL, #3300212B	1 vial
Std. C, 1.5 ml, 12.5 GPL/MPL-U/mL, #3300212C	1 vial
Std. D, 1.5 ml, 25 GPL/MPL-U/mL, #3300212D	1 vial
Std. E, 1.5 ml, 50 GPL/MPL-U/mL, #3300212E	1 vial
Std. F, 1.5 ml, 100 GPL/MPL-U/mL, #3300212F	1 vial
Positive Control, 1.5 ml#3300210P	1 vial
Negative Control, 1.5 ml#3300210N	1 vial
lot sp. Conc. mentioned on the each vial	
Sample Buffer (5X) 20 ml, #3300213	1 bottle
Enzyme Conjugate (G), 15 ml, Anti-hIgG#3300214G	1 bottle
Enzyme Conjugate (M), 15 ml, Anti-hIgM#3300214M	1 bottle
HRP **Substrate Solution**, 15 ml # #3300210TM	1 bottle
Wash buffer (50X), 20 ml, dilute 1:50 with distilled water #3300210-WB	1 bottle
Stop solution (ready-to-use), 15 ml, #3300210-ST	1 bottle
Complete Instruction Manual, M-3300-210-PSS	1

Intended Use

Anti-Phosphatidyl Serine is an indirect solid phase enzyme immunoassay (ELISA) for the determination of IgG and IgM class autoantibodies against phosphatidyl serine in human serum or plasma. ADI's Anti- Phosphatidyl Serine ELISA KIT is intended for research use only, not for use in diagnostic procedures.

General Information

Phosphatidylserine (PS) is a phospholipid component, on the cytosolic side of cell membranes by an enzyme called flippase. When a cell undergoes apoptosis, phosphatidylserine becomes exposed on the surface of the cell. Antiphospholipid syndrome or antiphospholipid antibody syndrome (APS or APLS or), often also Hughes syndrome, is an autoimmune, hypercoagulable state caused by antiphospholipid antibodies. APS provokes blood clots (thrombosis) in both arteries and veins as well as pregnancy-related complications such as miscarriage, stillbirth, preterm delivery, or severe preeclampsia.

PERFORMANCE CHARACTERISTICS

Specificity:

The microplate is coated with highly purified Phosphatidyl Serine and saturated with human beta-2-glycoprotein I. Special coating processes, developed by the manufacturer guarantee for the native immunogenic structure of the phospholipids after immobilization on the solid phase. The ELISA kit is specific for autoantibodies directed against the respective phospholipid or the complex of the negatively-charged phospholipid and beta-2- Glycoprotein I.

No cross reactivity was observed to anti-DNA antibodies and those types of antibodies occurring in syphilis.

Calibration:

The assay system is calibrated against the internationally recognized reference sera from E. N. Harris, Louisville, since no other international standards are available.

LIMITATIONS OF PROCEDURE

The Anti-Phosphatidyl Serine IgG/IgM ELISA is intended for research use only – not for use in diagnostic procedures. .

Interferences:

No interference has been observed with hemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera. Nor have any interfering effects been observed with the use of anticoagulants. However for practical reasons it is recommended that grossly hemolysed or lipemic samples should be avoided.

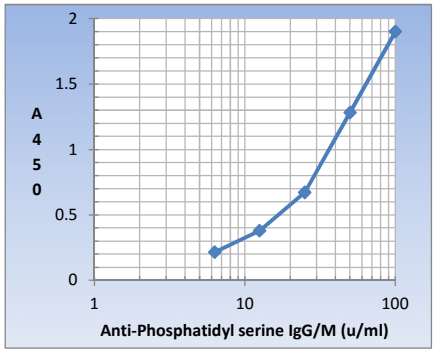
References:

Reynaud Q (2014) Autoimmune Red. In press; Saadatnia M (2007) Neurosci. 12, 124-126; Coulam CB (1997) J. Assist. Reprod. Genet. 14, 603-608; Arnold LW (1993) Intl. Immunol. 5, 1365-1373; Colaco CB (1985) Clin. Exp. Immunol. 59, 449-456; Triplett DA (2002) Arch. Pathol. Lab. Med. 126, 1424-1429; Miyakis (2006) J. Throm. Res. 4, 295-306;

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples MPL U/mL	Mean A_{450nm}
A1, A2	**Std. A** (0)	0.015
B1, B2	**Std. B** (6.3)	0.215
C1, C2	**Std. C** (12.5)	0.379
D1, D2	**Std. D** (25)	0.672
E1, E2	**Std. E** (50)	1.282
F1, F2	**Std. F** (100)	1.901

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



/3_ADI_arif/3300-210

A typical std. assay curve (do not use this for calculating sample values)

CALCULATION OF RESULTS:

This test is only valid if the optical density at 450 nm for positive control (1) and negative control (2) as well as for the calibrators A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit! If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

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.

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.

The diagnostic criteria requires one clinical event, i.e. thrombosis or pregnancy complication, and two positive blood tests spaced at least 3 months apart. These antibiodies are: lupus anticoagulant, anti-cardiolipin and anti-β2-glycoprotein-I.

The term "primary antiphospholipid syndrome" is used when APS occurs in the absence of any other related disease. APS however also occurs in the context of other autoimmune diseases, such as systemic lupus erythematosus (SLE), in which case the term "secondary antiphospholipid syndrome" is used. In rare cases, APS leads to rapid organ failure due to generalised thrombosis; this is termed "catastrophic antiphospholipid syndrome" (CAPS) and is associated with a high risk of death.

Antiphospholipid syndrome often requires treatment with anticoagulant medication such as heparin to reduce the risk of further episodes of thrombosis and improve the prognosis of pregnancy. Warfarin/Coumadin is not used during pregnancy because it can cross the placenta, unlike heparin, and is teratogenic.

PRINCIPLE OF THE TEST

Highly purified phosphatidyl serine is bound to microwells saturated with beta-2-glycoprotein I. antibodies against these antigens, if present in diluted serum or plasma, bind to respective antigens. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG and IgM immunologically detect the bound antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyses 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 color is directly proportional to the concentration of IgG resp. IgM antibodies present in the original sample.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (25-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.
TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).
All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).
Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

REAGENTS PREPRATION:

Dilute wash buffer 20 ml with 980 ml distilled water**.** Store at 4oC.

Dilute Sample Buffer (5X): 20 ml with 80 ml distilled water.

Sample preparation:

Dilute all 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.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually six months from the date of shipping, under appropriate storage conditions.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).* **Dilute wash buffer & Sample Buffer as per detail on page 2 before use.**

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. Label or mark the microtiter well strips to be used on the plate. Reference wells for identification.

2. Pipet **100 µl of standards**, control & pre-diluted patient samples, in appropriate wells in *duplicate*. Cover the plate and incubate for **30 minutes** at 20-28 oC.

3. Aspirate and wash the wells **3 times** with 300 µl wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Pipet **100 µl** Conjugate to each well. Cover the plate and incubate for **15 minutes** at 20-28 oC.

5. Aspirate and wash the wells **3 times** with 300 µl wash buffer as above.

6. Dispense **100 µl TMB substrate per well**. Mix gently for 5-10 seconds.

7. Cover the plate and incubate for **15 minutes** at 20-28 oC

8. Stop the reaction by adding **100 µl** of stopping solution to all wells. Mix gently for 5-10 seconds. Blue color turns yellow. Read the plate at 450 nm within 30 min.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{o}C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. do not touch the bottom of the wells.

QUALITY CONTROL

Each laboratory should utilize controls at several levels to monitor assay performance. The controls should be treated as unknown. Values obtained should be in a agreement with the assigned values of the control. Controls can be obtained from commercially available sources but should not contain sodium azide as preservative.