

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-3300-215-PIS

Anti-Phosphatidyl Inositol IgG/IgM

ELISA KIT Cat. # 3300-215-PIS

For the determination of IgG and IgM class Autoantibodies
against Phosphatidyl Inositol

For In Vitro Research Use Only



**ALPHA DIAGNOSTIC
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**DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE
KIT FOR ANY LOT SPECIFIC CHANGES.**

Anti- Phosphatidyl Inositol ELISA KIT Cat. No. 3300-215-PIS

Kit Contents: (reagents for 96 tests)

C o m p o n e n t s	
Highly purified phosphatidyl inositol and saturated with beta-2-glycoprotein I coated microwell strip plate (96 wells);#3300216	96 wells (1 plate)
Std. A , 1.5 ml, 0 GPL/MPL-U/mL, #3300217A	1 vial
Std. B , 1.5 ml, 6.3 GPL/MPL-U/mL, #3300217B	1 vial
Std. C , 1.5 ml, 12.5 GPL/MPL-U/mL, #3300217C	1 vial
Std. D , 1.5 ml, 25 GPL/MPL-U/mL, #3300217D	1 vial
Std. E , 1.5 ml, 50 GPL/MPL-U/mL, #3300217E	1 vial
Std. F , 1.5 ml, 100 GPL/MPL-U/mL, #3300217F	1 vial
Positive Control, 1.5 ml #3300215P (see lot sp. conc on the vial)	1 vial
Negative Control, 1.5 ml #3300215N (see lot sp. conc on the vial)	1 vial
Sample Buffer (5X) 20 ml, #3300218	1 bottle
Enzyme Conjugate(G) , Anti-hIgG, 15 ml, #3300219G	1 bottle
Enzyme Conjugate(M) , Anti-hIgM 15 ml, #3300219M	1 bottle
HRP Substrate Solution , 15 ml # #3300215TM	1 bottle
Wash buffer (50X) , 20 ml, dilute 1:50 with distilled water #3300215-WB	1 bottle
Stop solution (ready-to-use) , 15 ml, #3300215-ST	1 bottle
Complete Instruction Manual, M-3300-215-PIS	1

Intended Use

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

General information

Phosphatidylinositol is an important lipid, both as a key membrane constituent and as a participant in essential metabolic processes in all plants and animals, both directly and via a number of metabolites. It is an acidic (anionic) phospholipid that in essence consists of a phosphatidic acid backbone, linked via the phosphate group to inositol (hexahydroxycyclohexane). In most organisms, the stereochemical form of the last is myo-D-inositol (with one axial hydroxyl in position 2 with the remainder equatorial), although other forms (scyllo- and chiro-) have been found on occasion in plants. The 1-stearoyl,2-arachidonoyl molecular species, which is of considerable biological importance in animals, is illustrated.

Phosphatidylinositol is especially abundant in brain tissue, where it can amount to 10% of the phospholipids, but it is present in all tissues and cell types. There is usually less of it than of phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine.

PERFORMANCE CHARACTERISTICS

Specificity:

The microplate is coated with highly purified Phosphatidyl inositol 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 inositol IgG/IgM ELISA is intended for research use only – not for use in diagnostic procedures. .

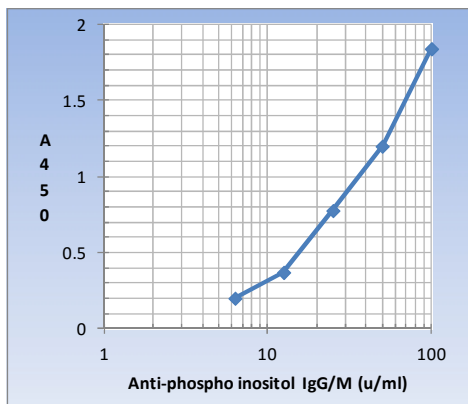
Interferences:

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.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples MPL U/mL	Mean A _{450nm}
A1, A2	Std. A (0)	0.009
B1, B2	Std. B (6.3)	0.020
C1, C2	Std. C (12.5)	0.37
D1, D2	Std. D (25)	0.77
E1, E2	Std. E (50)	1.20
F1, F2	Std. F (100)	1.84

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_ariff/3300-215

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 inositol 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.

PRINCIPLE OF THE TEST

Highly purified phosphatidyl inositol 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 Proclin-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).

REAGENTS PREPRATION:

Dilute wash buffer 20 ml with 980 ml distilled water. Store at 4°C.

Dilute Sample Buffer (5X) 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°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.

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.

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.

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°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.