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

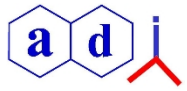
Instruction Manual No. M-3300-200-B2A

Human Anti-Beta2-Glycoprotein 1 IgA

ELISA KIT Cat. No. 3300-200-B2A

for the detection of human IgA class autoantibodies against beta 2 glycoprotein (β2GP1) in human serum or plasma

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.

Human Anti-Beta2-Glycoprotein 1 IgA cat# 3300-200-B2A

Kit Contents: (reagents for 96 tests)

Components	
β2GP1 Antigen coated microwell strip plate (96 wells);#3300201	96 wells (1 plate)
Anti- β2GP1 IgA **Std. A**, 1.5 ml, 0 U/mL, #3300202A	1 vial
Anti- β2GP1 IgA **Std. B**, 1.5 ml, 6.3 U/mL, #3300202B	1 vial
Anti- β2GP1 IgA **Std. C**, 1.5 ml, 12.5 U/mL, #3300202C	1 vial
Anti- β2GP1 IgA **Std. D**, 1.5 ml, 25 U/mL, #3300202D	1 vial
Anti- β2GP1 IgA **Std. E**, 1.5 ml, 50 U/mL, #3300202E	1 vial
Anti- β2GP1 IgA **Std. F**, 1.5 ml, 100 U/mL, #3300202F	1 vial
Anti- β2GP1 IgA **Positive Control, 1.5 ml**#3300202P	1 vial
Anti- β2GP1 IgA **Negative Control, 1.5 ml**#3300202N	1 vial
lot sp. Conc. mentioned on each vial	
Sample Buffer (5X) 20 ml, #3300203	1 bottle
Anti-Rabbit IgA **HRP Conjugate**, 15 ml, #3300204	1 bottle
HRP **substrate Solution**, 15 ml # #3300200TM	1 bottle
Wash buffer (50X), 20 ml, dilute 1:50 with distilled water #3300200-WB	1 bottle
Stop solution (ready-to-use), 15 ml, #3300200-ST	1 bottle
Complete Instruction Manual, M-3300-200-B2A	1

Intended Use

ADI's Anti-Beta2-Glycoprotein-1 IgA ELISA KIT an indirect solid phase enzyme immunoassay (ELISA) for the determination of IgM class autoantibodies against β2GP 1 IgA in human serum or plasma. ELISA KIT is intended for research use only, not for use in diagnostic procedures.

General Information

β2-glycoprotein 1 (β2GP1) is a plasma glycoprotein and has been shown to be the dominant antigen for anti-phospholipid antibodies (APAs) in patients with anti-phospholipid syndrome. The presence of APAs such as anti-cardiolipin antibodies is associated with venous and arterial thrombosis, recurrent spontaneous abortions and thrombocytopaenia. Anti-cardiolipin antibodies are also present in response to a variety of infections and certain drug treatments. A number of studies have demonstrated that detection of β2GP1 antibodies, in conjunction with anti-cardiolipin measurement, is important in defining the thrombotic risk associated with APAs. β2GP1 IgG-antibodies are more closely associated with thrombosis in patients with a history of anti-phospholipid antibody-associated diseases than anti-cardiolipin antibodies.

INTERPRETATION OF RESULTS:

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti- β2-glycoprotein I tests:

	Anti-β2-Glycoprotein I-Ab
normal:	< 5
borderline:	5 - 8
elevated:	> 8

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti-b2-glycoprotein I. The values below should be regarded as guidelines only.

PERFORMANCE CHARACTERISTICS

1. PRECISION

Statistics for Coefficients of variation (CV) were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 3 different runs with 16 determinations of each sample:

Intra-assay precision:

Sample	Mean Value U/mL	%CV
1	3.6	3.0
2	8.5	3.2
3	13.4	2.5

Inter-assay precision:

Sample	Mean Value U/mL	%CV
1	6.6	7.4
2	12.1	8.5
3	28.3	7.7

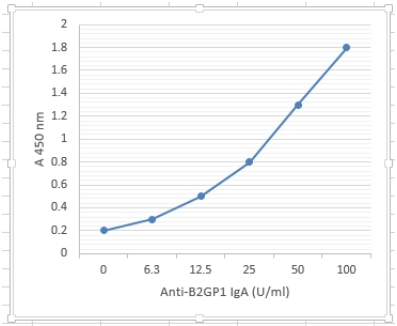
References:

1. Roubey, R.A.S. Review Article: Autoantibodies to phospholipid-binding plasma proteins: a new view of Lupus Anticoagulants and other "antiphospholipid" autoantibodies. Blood 1994; Vol 84, No 9: 2854 - 2867.
2. Schousboe, I. b2-Glycoprotein I: a plasma inhibitor of the contact activation of the intrinsic blood coagulation pathway. Blood 1985; Vol 66, No 5: 1086 - 1091.
3. Lee, N.S. et al. b2-Glycoprotein I - Molecular properties of an unusual apolipoprotein, Apolipoprotein H. J. Biol. Chem. 1983; Vol 258, No 8: 4765 - 4770.
4. Kandiah, D.A. et al. Epitope mapping studies of antiphospholipid antibodies and b2GPI using synthetic peptides. Lupus 1995; Vol 4, Suppl 1: S7 - S11.
5. Matsuura, E. et al. Molecular studies on phospholipid-binding sites and cryptic epitopes appearing on b2-glycoprotein I structure recognized by anti-cardiolipin antibodies. Lupus 1995; Vol 4, Suppl 1: S13 - S17.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples U/mL	Mean A_{550nm}
A1, A2	**Std. A** (0)	
B1, B2	**Std. B** (6.3)	
C1, C2	**Std. C** (12.5)	
D1, D2	**Std. D** (25)	
E1, E2	**Std. E** (50)	
F1, F2	**Std. F** (100)	

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.



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CALCULATION OF RESULTS:

For Anti-β2-glycoprotein I IgA a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed Spline Approximation and log-log coordinates are also suitable.

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.

Quality Control

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 Std 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 met, the results are invalid and the test should be repeated.

PRINCIPLE OF THE TEST

The wells of the microtitre strips are coated with highly purified human β2GP1 IgA antigen. During the first incubation, specific autoantibodies in diluted serum or plasma bind to the antigen-coated surface; the wells are then washed to remove unbound components. In the second incubation, the Conjugate, enzyme-labelled antibodies to human IgA, binds any surface-bound autoantibodies. After further washing, specific autoantibodies are traced by incubation with the Substrate. Addition of Stop Solution terminates the reaction, resulting in a colored end-product. The amount of Conjugate bound is measured in absorbance units. In the qualitative protocol, the amount of Conjugate bound by the sample is compared with that bound by the Reference Control. In the quantitative protocol, the concentration of anti-β2GP1 IgA autoantibody can be estimated by interpolation from a dose-response curve based on Standards.

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.

LIMITATIONS

1.The Alpha Diagnostic International ELISA test is intended for *in vitro research* use only.
Although the presence of high titres of aCL antibodies is associated with clinical symptoms the information is an aid to diagnosis only, and must be considered in light of other clinical and laboratory findings.
2. If a current or prior syphilis infection is suspected this should be confirmed or ruled out by a specific test for anti-treponemal antibodies, as the patient may have a positive result without increased risk of thrombosis.
3. Low to moderate levels of aCL antibodies have been reported in acute infection (32%)10, asymptomatic elderly patients (2-52%)4-7, and healthy blood donors (2%)4. In the majority of cases, these conditions are not reported to be accompanied by thrombotic events, and clinical interpretation is unclear. If such patients test positive while there are clinical signs, e.g. infection, the test should be repeated after six months.

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 1000 ml final volume of distilled water. Store at 4oC.

Dilute Sample Buffer (5X): 25 ml with 100 ml distilled water.

SAMPLE PREPARATION : Dilute all patient 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.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).* **Dilute wash buffer & Sample Diluent as per detail as above.**

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 controls** and pre-diluted patient samples and, in appropriate wells in *duplicate*. into appropriate wells. 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** enzyme Conjugate to each well. Cover the plate and incubate for 1**5 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.

SENSITIVITY

The lower detection limits for anti-β2-Glycoprotein I IgG and IgM were determined at 0.5 U/ml.

SPECIFICITY

The microplate is coated with highly purified human β2-Glycoprotein I. The test kit is specific only for autoantibodies against β2-Glycoprotein I. Endogenous β2-Glycoprotein I and endogenous negatively-charged phospholipids occur in (1:100)-diluted samples at approx. 2 µg/ml and approx. 1 µg/ml, respectively. Influences on the determination of anti-β2-Glycoprotein I -antibodies have not been observed.

CALIBRATION

Since no international reference preparation for anti-β2-Glycoprotein I autoantibodies is available, the assay system is calibrated in relative arbitrary units. The calibration is related to the internationally recognized reference sera from E.N. Harris, Louisville. These sera test positive for Anti-β2-Glycoprotein I autoantibodies.

LIMITATIONS OF PROCEDURE

The Anti-β2-Glycoprotein I IgA ELISA is a diagnostic aid and by itself is not diagnostic. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

INTERFERING SUBSTANCES

No interference has been observed with haemolytic (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 hemolyzed or lipemic samples be avoided.