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

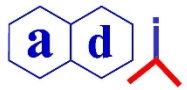
Instruction Manual No. M-3300-195-B2M

Human Anti-Beta2-Glycoprotein 1 IgM

ELISA KIT Cat. No. 3300-195-B2M

for the detection of human IgM 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 IgM cat# 3300-195-B2M

Kit Contents: (reagents for 96 tests)

Components	
β2GP1 Antigen coated microwell strip plate (96 wells);#3300196	96 wells (1 plate)
Anti- β2GP1 IgM **Std. A**, 1 ml, 0 U/mL, #3300197A	1 vial
Anti- β2GP1 IgM **Std. B**, 1 ml, 2 U/mL, #3300197B	1 vial
Anti- β2GP1 IgM **Std. C**, 1 ml, 8 U/mL, #3300197C	1 vial
Anti- β2GP1 IgM **Std. D**, 1 ml, 30 U/mL, #3300197D	1 vial
Anti- β2GP1 IgM **Std. E**, 1 ml, 100 U/mL, #3300197E	1 vial
Anti- β2GP1 IgM **Ref. Control**, 1.5 ml, #3300197RC	1 vial
Anti- β2GP1 IgM **Positive Control, 0.2 ml**#3300195P	1 vial
Anti- β2GP1 IgM **Negative Control, 0.1 ml**#3300195N	1 vial
lot sp. Conc. mentioned on each vial	
Anti- β2GP1 **Sample Diluent (5X)** 25 ml, #3300198	1 bottle
Anti- β2GP1 IgM **Conjugate**, 15 ml, #3300199	1 bottle
HRP **substrate Solution**, 15 ml # #3300195TM	1 bottle
Wash buffer (16X), 2X 25 ml, dilute 1:16 with distilled water #3300195-WB	1 bottle
Stop solution (ready-to-use), 15 ml, #3300195-ST	1 bottle
Complete Instruction Manual, M-3300-195-B2M	1

Intended Use

ADI's Anti-Beta2-Glycoprotein IgM ELISA KIT an indirect solid phase enzyme immunoassay (ELISA) for the determination of IgM class autoantibodies against β2GP1 in human serum or plasma or citrate/EDTA anticoagulated 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.

Clinical Data:

Primary anti-Phospholipid Syndrome: 35 samples from clinically diagnosed PAPS were analyzed; 16 were positive for β2GP1 IgM

Viraemia: 16 samples were analyzed; one was positive for β2GP1 IgM.

Syphilis: 52 samples were analyzed; all were negative for β2GP1 IgM except for two.

PERFORMANCE CHARACTERISTICS

1. PRECISION

Intra-assay precision: *determine by four samples, with replication of twelve.*

Sample	Mean Value U/mL	%CV
1	3.6	3.0
2	8.5	3.2
3	13.4	2.5
4	39.4	3.5

Inter-assay precision: *determine by four samples, in 20 assays using three kits batches & 20 discrete sample dilutions..*

Sample	Mean Value U/mL	SD	%CV
1	6.6	0.49	7.4
2	12.1	1.03	8.5
3	28.3	2.19	7.7
4	31.4	2.63	8.4

Detection Limit

The lower limit of detection, calculated as the mean of the zero standard plus two standard deviations, run in duplicate in 20 assays was 0.1 U/mL.

Interferences:

Haemolysate up to 400mg/dl, bilirubin up to 0.2mg/mL and intralipid up to 15mg/mL do not interfere with results.

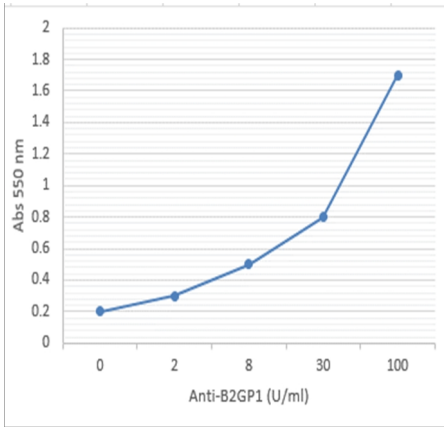
References:

1. McNally T, Mackie IJ, et al. Blood Coagulation and Fibrinolysis, 6, 411-416, 1995.
2. McNally T, Mackie IJ, et al. Thrombosis & Haemostasis, 76, 220-225, 1996.
3. Matsuura E and Koike T. Autoantibodies, Eds. Peter JB and Shoenfeld Y, Elsevier, Amsterdam, 109-114, 1996.
4. Khamashta MA and Hughes GRV. Autoantibodies, Eds. Peter JB and Shoenfeld Y, Elsevier, Amsterdam, 624-629, 1996.
5. Viard JP, Amoura Z, and Bach JF. Am J Med, 93, 181-186, 1992.
6. McNally T, Mackie IJ, et al. Br J Rheum, 34, 1031-1036, 1995.
7. Martinuzzo ME, Forastiero RR and Carreras LO. Br J Haem, 89, 397-402, 1995.
8. McNally T, Purdy G, et al. Br J Haem, 91, 471-473, 1995.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples U/mL	Mean A_{550nm}
A1, A2	**Std. A** (0)	
B1, B2	**Std. B** (2)	
C1, C2	**Std. C** (8)	
D1, D2	**Std. D** (30)	
E1, E2	**Std. E** (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:

Qualitative Protocol:

Calculate the absorbance ratio for each sample and Positive and Negative Controls.

Absorbance Ratio = Sample **or** Control Absorbance Value
mean Reference Control Absorbance Value

Ratios	Suggested Interpretation
<=1.0	Negative
>1.0 to 1.2	Borderline- A repeat test should be carried out on a subsequent sample. Note that the clinical significance of borderline levels of antibodies is the subject of debate, and these results should be considered in light of other diagnostic and clinical information.
>1.2	Positive

Anti-phospholipid syndrome (APS) can be primary, or secondary to other diseases, most commonly systemic lupus erythematosus (SLE)5. aCL are also observed in patients without APS, particularly syphilis patients. In addition, moderately elevated aCL levels can be observed in the normal population, typically with a reported incidence between 1 and 8%5. In general, aCL originating from infections tend not to be associated with any clinical symptoms..

PRINCIPLE OF THE TEST

The wells of the microtitre strips are coated with highly purified human β2GP1 IgM 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 IgM, 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 IgM 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: Do not dilute Ref. Control.

Dilute wash buffer 25 ml with 375 ml distilled water. Store at 4oC.
Dilute Sample Diluent (5X): 25 ml with 100 ml distilled water.
Positive & Negative controls/samples 1:101 (10 µl with **1 ml 1x Sample Diluent**)

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 Diluent as per detail above.**

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

Qualitative protocol: run Reference Control, Positive and Negative Controls, and samples.

Quantitative protocol: run Standards (A-E), Positive and Negative Controls, and samples.

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

2. Pipet **100 µl of standards**, Ref control, in appropriate wells in *duplicate*. pre-diluted Positive and Negative Controls, and pre-diluted patient samples(1:101) into appropriate wells. Remember to change pipette tips between additions. This step should not exceed 15 minutes for any one set of Standards/Controls/samples. Cover the plate and incubate for **60+-10 minutes** at 18-25 oC.

3. Aspirate and wash the wells **3 times** with **200 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 IgM** Conjugate to each well. Cover the plate and incubate for **30+-5 minutes** at 18-25 oC.

5. Aspirate and wash the wells 3 **times** with 200 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 **30+-5 minutes** at 18-25 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 550 nm(540-565 nm) within 30 min.

Expected Values:

148 serum samples from asymptomatic apparently healthy donors, an almost equal ratio of male to female, aged between 17 and 77 years, were assayed for anti-β2GP1 IgM. It is recommended that users establish reference ranges for the populations served by their own laboratories; the following is only intended as a guide to the interpretation of results.

Qualitative Protocol:

Ratios	Suggested Interpretation
<=1.0	Negative
>1.0 to 1.2	Borderline- A repeat test should be carried out on a subsequent sample. Note that the clinical significance of borderline levels of antibodies is the subject of debate, and these results should be considered in light of other diagnostic and clinical information.
>1.2	Positive

It is recommended that positive samples are re-assayed using the quantitative protocol.

Semi-Quantitative Protocol:

Concentration U/mL	Suggested Interpretation
<6	Negative
>6 to <=8	Equivocal results-repeat testing, recommended on a fresh dilution of the sample and/or obtain a further sample at an appropriate interval.
>8	Positive

Semi-Quantitative Protocol:

Calculate the mean absorbance value of each Standard and plot against log10 Standard concentration (see following table) on suitable graph paper. The concentration of samples and Controls can then be read from the standard curve; a typical plot is shown below for reference purposes, it must not be used for interpreting results Smooth spline, weighted 4- or 5-parameter logistic, log/logit, or lin/linit are also satisfactory.

Samples with absorbances above Standard E (100 U/mL) are outside the range of the assay, and should be reported as >100 U/mL, diluted and re-assayed, correcting for the dilution factor.
NB: As in any assay measuring antibodies, this assay determines the activity of the antibody present in the sample, not the concentration. Activity can be affected by a number of parameters, such as antibody activity.