Human Anti-Beta2-Glycoprotein 1 IgG ELISA KIT Cat. No. 3300-190-B2G

for the detection of human IgG class autoantibodies against beta 2 glycoprotein (B2GP1) in human serum or plasma

For In Vitro Research Use Only

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, Quantitative
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-Sci-70 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG ELISA kit,
3300-150-JCG Human Anti-Jo-1 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG ELISA kit, 96
3300-160-APG Human Anti-Alpha Fetin 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
3300-180-CLA Human Anti-Cardiolipin IgA ELISA kit, 96 tests, Quantitative
3300-190-B2G Human Anti-Beta2-Glycoprotein 1 IgG ELISA kit, 96 tests, Quantitative
3300-200-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 1) IgG/IgM ELISA kit, 96 tests, Quantitative
3300-210-PSS Human Anti-Phosphatidyl serine IgG/IgM ELISA kit, 96 tests, Quantitative
3300-215-PIS Human Anti-Phosphatidyl Inositol IgG/IgM ELISA kit, 96 tests, Quantitative
3300-220-PAS Human Anti-Phosphatidic 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, Quantitative
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-bacterial 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
3300-310-PREG Human Anti-Helical Parietal cell (alpha and beta subunits of the Parietal Cell
(H/KATPase) 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
Human Anti-Beta2-Glycoprotein 1 IgG cat# 3300-190-B2G

Kit Contents: (reagents for 96 tests)

<table>
<thead>
<tr>
<th>Components</th>
<th>96 wells (1 plate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β2GP1 Antigen coated microwell strip plate (96 wells): #3300191</td>
<td>96 wells (1 plate)</td>
</tr>
<tr>
<td>Anti-β2GP1 IgG Std. A, 1 ml, 0 U/mL, #3300192A</td>
<td>1 vial</td>
</tr>
<tr>
<td>Anti-β2GP1 IgG Std. B, 1 ml, 2 U/mL, #3300192B</td>
<td>1 vial</td>
</tr>
<tr>
<td>Anti-β2GP1 IgG Std. C, 1 ml, 8 U/mL, #3300192C</td>
<td>1 vial</td>
</tr>
<tr>
<td>Anti-β2GP1 IgG Std. D, 1 ml, 30 U/mL, #3300192D</td>
<td>1 vial</td>
</tr>
<tr>
<td>Anti-β2GP1 IgG Std. E, 1 ml, 100 U/mL, #3300192E</td>
<td>1 vial</td>
</tr>
<tr>
<td>Anti-β2GP1 IgG Ref. Control, 1.5 ml, #3300192RC</td>
<td>1 vial</td>
</tr>
<tr>
<td>Anti-β2GP1 IgG Positive Control, 0.2 ml#3300190P</td>
<td>1 vial</td>
</tr>
<tr>
<td>Anti-β2GP1 IgG Negative Control, 0.1 ml#3300190N</td>
<td>1 vial</td>
</tr>
<tr>
<td>Anti-β2GP1 IgG Diluent (5X) 25 ml, #3300193</td>
<td>1 vial</td>
</tr>
<tr>
<td>Anti-β2GP1 IgG Conjugate, 15 ml, #3300194</td>
<td>1 vial</td>
</tr>
<tr>
<td>HRP substrate Solution, 15 ml #3300190TM</td>
<td>1 vial</td>
</tr>
<tr>
<td>Wash buffer (16X), 2X 25 ml, dilute 1:16 with distilled water #3300190-WB</td>
<td>1 bottle</td>
</tr>
<tr>
<td>Stop solution (ready-to-use), 15 ml, #3300190-ST</td>
<td>1 bottle</td>
</tr>
<tr>
<td>Complete Instruction Manual, M-3300-190-B2G</td>
<td>1</td>
</tr>
</tbody>
</table>

Intended Use

ADI’s Anti-Beta2-Glycoprotein IgG ELISA KIT an indirect solid phase enzyme immunoassay (ELISA) for the determination of IgG 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 thrombocytopenia. 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: 43 samples from clinically diagnosed PAPS were analyzed; 30 were positive for aCL autoantibodies

Viraemia: 14 samples were analyzed; all were negative for β2GP1 IgG autoantibodies

Syphils: 64 samples were analyzed; all were negative for β2GP1 IgG autoantibodies

PERFORMANCE CHARACTERISTICS

1. PRECISION

Intra-assay precision: determine by three samples, in 20 assays using three operators & using three kits batches..

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Value U/mL</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.2</td>
<td>8.6</td>
</tr>
<tr>
<td>2</td>
<td>12.1</td>
<td>7.5</td>
</tr>
<tr>
<td>3</td>
<td>25.2</td>
<td>8.2</td>
</tr>
</tbody>
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<tr>
<td>1</td>
<td>6.2</td>
<td>11.6</td>
</tr>
<tr>
<td>2</td>
<td>12.1</td>
<td>9.7</td>
</tr>
<tr>
<td>3</td>
<td>25.2</td>
<td>11.5</td>
</tr>
</tbody>
</table>

Interferences:

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

References:

Anti-phospholipid syndrome (APS) can be primary, or secondary to other diseases, most commonly systemic lupus erythematosus (SLE). aCL are also observed in patients without APS, particularly syphils patients. In addition, moderately elevated aCL levels can be observed in the normal population, typically with a reported incidence between 1 and 8%. 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 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 IgG, 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 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%) to 10% asymptomatic elderly patients (2-52%) and healthy blood donors (2%). 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).

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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

Alpha Diagnostic Intl (www.4adi.com)

3300190B2G/151111A
REAGENTS PREPARATION: Do not dilute Ref. Control.

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

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 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 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°C.
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 Conjugate to each well. Cover the plate and incubate for 30±5 minutes at 18-25°C.
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°C
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:

152 serum samples comprising an almost equal ratio of male to female, aged between 18 and 68 years, were assayed for anti-β2GP1 antibodies. Two sample values (each 14.2 U/ml) fell out with mean plus five standard dev. of the population, and were omitted from calculation of the reference range. The overall mean of 150 samples was 2.82+1.46 U/ml. 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:

<table>
<thead>
<tr>
<th>Ratios</th>
<th>Suggested Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.8</td>
<td>Negative</td>
</tr>
<tr>
<td>&gt;=0.8   to &lt;=1.0</td>
<td>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.</td>
</tr>
<tr>
<td>&gt;1.0</td>
<td>Positive</td>
</tr>
</tbody>
</table>

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

Quantitative Protocol:

<table>
<thead>
<tr>
<th>Concentration U/ml</th>
<th>Suggested Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>Negative</td>
</tr>
<tr>
<td>&gt;10 to 15</td>
<td>Equivocal result.</td>
</tr>
<tr>
<td>&gt;15</td>
<td>Positive</td>
</tr>
</tbody>
</table>

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/logit 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.