Human beta2-Glycoprotein I antibody IgG / IgM ELISA Kit

Enzyme Immunoassay for the quantification of IgG/IgM antibodies to β2-Glycoprotein I in serum or plasma.

Catalog number: ARG80419

For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION **Page**

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INTRODUCTION

Anti phospholipid syndrome (APS, Hughes Syndrome) is a systemic autoimmune disease that causes thromboses, recurrent miscarriage, and intrauterine foetal death. Clinical symptoms are accompanied by the occurrence of specific autoantibodies that are detectable in the blood of patients with APS. These antibodies bind to phospholipids like cardiolipin, or phospholipid-binding proteins like beta-2-glycoprotein I. The clinical symptoms of APS alone are not sufficiently specific to make a definitive diagnosis. Laboratory tests thus play an important role in the diagnosis of the disease. The Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis defined clinical criteria and diagnostically relevant laboratory parameters in the Sapporo Criteria for the classification of anti phospholipid syndrome, published in 1999. These were revised and updated in 2006 and 2012.

They include the following laboratory parameters:

1. Detection of lupus anticoagulant (LA) in the plasma twice in the space of twelve weeks, according to the guidelines of the International Society on Thrombosis and Hemostasis.
2. Elevated anti-cardiolipin titre (IgG and/or IgM) in the blood. The values must be determined on two occasions at least twelve weeks apart using standardized ELISA tests for beta-2-glycoprotein I dependent cardiolipin antibodies.
3. Elevated beta-2-glycoprotein I antibody titre (IgG and/or IgM). The values must be determined on two occasions at least twelve weeks apart. Detection is performed by means of a standardized ELISA test.

The diagnosis of APS is considered as confirmed when at least one clinical and one of

the laboratory criteria are fulfilled. In primary APS autoantibodies against phospholipids appear independently, while in secondary APS phospholipid antibodies are detected in conjunction with other autoimmune diseases, such as lupus erythematosus, rheumatoid arthritis, or Sjögren's syndrome. Phospholipid antibodies are detectable in only 1-5 % of healthy individuals, but they are found in 16-35 % of lupus patients.

The presence of anti-cardiolipin antibodies in systemic lupus erythematosus (SLE) can be related to the development of thrombosis and thrombocytopenia. In gynaecology they are supposed to cause intrauterine death or recurrent abortion. Furthermore, anti-cardiolipin antibodies have been detected in neurological disorders like cerebrovascular insufficiency, cerebral ischemia, epilepsy or chorea. Anticardiolipin autoantibodies occur in the immunoglobulin classes IgG, IgM or IgA. The determination of IgM antibodies is a valuable indicator in the diagnosis of beginning autoimmune diseases, whereas IgG antibodies are present in progressive stages of manifested autoimmune disorders. The determination of IgA antibodies seems to have a greater importance in the African-Caribbean population. Quantitative measurement of anti-cardiolipin antibodies, especially IgG, shows high specificity in therapymonitoring of secondary APS related to SLE. Clinical indications for determination of anti-cardiolipin antibodies are: SLE, thrombosis, thrombocytopenia, cerebral ischemia, chorea, epilepsy, recurrent abortion, intrauterine death. The discovery that anti-phospholipid antibodies recognize plasma proteins that are associated with phospholipids rather than binding to the phospholipids themselves has been a major advance in APS research. Several reports indicate that beta-2-glycoprotein I antibodies are clinically relevant. Recent studies suggest the presence of a dominant epitope on the first domain of beta-2-glycoprotein I. In contrast to antibodies recognizing other domains

of beta-2- glycoprotein I, anti-domain I antibodies are found to be highly associated with clinical symptoms. Anti-cardiolipin and anti-beta-2-glycoprotein I antibodies are independent risk factors for the occurrence of vascular thrombosis and pregnancy loss. However, patients testing positive for multiple antibody specificities generally have a more severe disease and higher recurrence rates despite treatment.

Besides the standardized laboratory assays for detection of anti-cardiolipin antibodies, antibodies directed to beta-2-gycoprotein I and LA, defined in the classification criteria, several other autoantibodies have shown to be relevant to APS. Among them are antibodies against negatively-charged phospholipids, like phosphatidyl serine, phosphatidyl inositol and phosphatidic acid (PA). These antigens can improve the clinical sensitivity in patient samples with suspected APS but they will not replace the determination of autoantibodies against cardiolipin or beta-2-glycoprotein I. Autoantibodies that bind to proteins of the coagulation cascade or complexes of these proteins with phospholipids have also been proposed to be relevant for APS. As an example, a test for anti-prothrombin antibodies in conjunction with other parameters may be a good risk marker for thrombosis. Antibodies to Annexin V may also be detectable within the clinical framework of APS with otherwise negative phospholipid antibody results.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. A highly purified Beta-2-Glycoprotein I has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Ab present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated anti human antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard A-F (Concentration indicated on vial label)	6 vials (Containing beta-2-glycoprotein I antibodies. Ready-to-use)	4°C
Controls	2 vials (Ready-to-use)	4°C
5X Sample buffer	20ml	4°C

HRP-Antibody conjugate, IgG	15ml (Ready-to-use)	4°C
HRP-Antibody conjugate, IgM	15ml (Ready-to-use)	4°C
50X Wash buffer	20ml	4°C
TMB substrate	15ml	4°C (Protect from light)
STOP solution	15ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer.
- 1X Sample buffer: Dilute 5X Sample buffer with distilled water before use.
- Patient sample: Dilute patient sample 1:100 with 1X sample buffer before assay, mix well. (e.g. 10 µl of sample + 990 µl of 1X sample buffer)

  Note: the controls and calibrators are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100µl of standards, controls, samples and zero controls into wells.
3. Incubate for 30 minutes at RT.
4. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100µl 1X Antibody solution into each well. Incubate for 15 minutes at RT.
6. Wash as according to step 4.
7. Add 100µl of TMB Reagent to each well. Incubate for 15 minutes at room temperature.
8. Add 100µl of Stop Solution to each well. Incubate for 5 minutes at RT. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

INTERPRETATION OF RESULTS

Negative: <5 U/ml (For both IgG or IgM)

Borderline: 5-8 U/ml (For both IgG or IgM)

Positive: >8 U/ml (For both IgG or IgM)

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of β2-Glycoprotein I Ab ranged from 6.3-100 U/ml. The mean MDD was 0.5 U/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3.3% and inter-assay precision was 6%. [IgG]

The CV value of intra-assay precision was 2.6% and inter-assay precision was 4.9%. [IgM]