



IMUCLONE® Anti-β₂GPI IgG ELISA

Product No. 618G

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INTENDED USE

The IMUCLONE® Anti-β₂GPI IgG ELISA is an enzyme-linked immunosorbent assay for measuring auto-antibodies to β₂GPI of the IgG isotype in human plasma, serum or any biological fluid where auto-antibodies to β₂GPI may be present. The ELISA is limited to Research Use Only in the United States.

PRINCIPLE OF THE METHOD

The IMUCLONE® Anti-β₂GPI IgG ELISA uses a highly purified native uncleaved β₂GPI for isolating the autoantibody to β₂GPI. A diluted plasma sample or biological fluid is added to a β₂GPI coated microwell. If present, anti-β₂GPI autoantibodies bind to the immobilized β₂GPI. Following a wash step, bound autoantibodies of the IgG isotype are detected using a peroxidase conjugated goat anti-human IgG (Fcγ specific) antibody, which reacts specifically with IgG isotypes. Following another wash step, the peroxidase substrate 3,3',5,5' – tetramethylbenzidine (TMB), in presence of hydrogen peroxide (H₂O₂), is added to the microwell and the subsequent enzymatic reaction yields a blue colored solution. Last, the addition of sulphuric acid stops the reaction and turns the solution color to yellow. The amount of color is directly proportional to the amount of anti-β₂GPI IgG autoantibodies present in the tested sample.

REAGENTS

- 12 strips of 8 β₂GPI coated microwells (total of 96 wells) in frame holder.
- 2 vials of Autoimmunity Sample Diluent, ready to use (50 mL).
- 3 vials of Anti-β₂GPI IgG Positive Control (lyophilized).
- 3 vials of Anti-β₂GPI IgG Negative Control (lyophilized).
- 3 vials of Anti-IgG (Fcγ)-HRP Immunoconjugate (lyophilized).
- 1 vial of Conjugate Diluent, ready to use (25 mL).
- 1 vial of Wash Solution, 20 fold concentrate (50 mL).
- 1 vial of TMB Substrate, ready to use (25 mL).
- 1 vial of Stop Solution, 0.45M H₂SO₄ (6 mL).

WARNING

Source material for some of the reagents in this kit is of human origin. This material has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA approved methods. As no known test method provides complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, reagents should be handled as recommended for any potentially infectious human specimen. Discard all waste associated with test specimens and human source reagents in a biohazard waste container.

Limited for research use only in the United States. For *in vitro* use only. Not for internal use in humans or animals. Do not use the kit components beyond the stated expiration date. Do not mix reagents from different kits. Avoid microbial contamination of the reagents. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Do not pipette reagents by mouth. Wear laboratory coat and disposable gloves throughout the test procedure and wash hands thoroughly afterwards. Avoid splashing or aerosol formation. Do not expose the TMB reagent to strong sunlight.

REAGENT PREPARATION AND STORAGE

Unopened and lyophilized reagents are stable until the expiration date printed on the box when properly stored at 2°-8°C.

- β₂GPI Coated Microwells:** Once removed from the aluminium pouch, the microwell strips must be used within 30 minutes. Unused strips may be stored at 2°-8°C for 4 weeks when sealed in the original pouch with the desiccant present, protected from any moisture, and stored in the provided storage bag.
- Autoimmunity Sample Diluent:** Supplied ready to use, once opened, the diluent may be used for up to 4 weeks when stored at 2°-8°C. **Warning:** The Autoimmunity Sample Diluent contains sodium azide that may react with lead and copper plumbing to form highly explosive metal azides. Flush with large volumes of water when discarding into a sink.
- Positive Control:** Reconstitute each vial with 1 mL of Autoimmunity Sample Diluent. This control is equivalent to plasma containing IgG isotype autoantibodies to β₂GPI at a 1:100 dilution. Reconstituted Positive Control is stable for 2 weeks at 2°-8°C providing bacterial contamination is avoided.
- Negative Control:** Reconstitute each vial with 1 mL of Autoimmunity Sample Diluent. This control is equivalent to a normal a 1:100 dilution. Reconstituted Negative Control is stable for 2 weeks at 2-8°C providing bacterial contamination is avoided.
- Anti-IgG (Fcγ)-HRP Immunoconjugate:** Reconstitute each vial with 7.5 mL of Conjugate Diluent. Shake the vial gently to homogenize the content. Reconstituted immunoconjugate is stable for at least 24 hours at room temperature or for at least 4 weeks at 2°-8°C.
- Conjugate Diluent:** Supplied ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C.
- Wash Solution:** If solids are present, incubate the vial for 15-30 minutes in a 37°C water bath. Shake the vial and dilute the amount required 1:20 in distilled water (the 50 mL is sufficient to prepare 1 Liter of Wash Solution). The Wash Solution may be used for up to 4 weeks after opening when stored at 2°-8°C in its original vial. Diluted Wash Solution may be used for up to 7 days when stored at 2°-8°C.
- TMB Substrate:** Supplied ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C.
- 0.45M H₂SO₄:** Supplied ready to use. **Caution:** Sulphuric acid, although diluted to 0.45M is caustic. As for any similar chemical, handle with great care. Avoid any skin and eye contact. Wear protection glasses and gloves when handling.

SPECIMEN COLLECTION AND PREPARATION

Either citrate or EDTA collected platelet poor plasma or serum may be used for this assay. Plasma collection should be performed as follows:

- Collect 9 parts of blood into 1 part of 3.2% (0.109M) trisodium citrate anticoagulant solution.
- Centrifuge the blood sample at 1,500 rpm for 15 minutes.
- Plasma should be stored at 2°-8°C and assayed within 4 hours. Alternatively, plasma may be stored at -20°C for up to 6 months.
- Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2°-8°C and assayed within 4 hours.

Plasma or serum is tested at 1:100 dilution in Autoimmunity Sample Diluent. When high amounts of autoantibodies to β₂GPI are expected, samples must be assayed at 1:200 or 1:400 dilution.

PROCEDURE

Materials Provided – See Reagents

Materials Required But Not Provided

- 0.22 μm filtered deionized H₂O
- 50-300 μL eight channel multi-pipette
- 0-200 μL, 200-1000 μL single pipettes
- Microwell plate reader for reading absorbance at 450 nm
- Microwell plate washer (optional)

Preparation of the Standards

The assay is calibrated using the Positive Control provided, the concentration of which (C) is indicated in arbitrary units, (AU) on the flyer provided. Standard solutions are prepared by performing serial dilutions of the Positive Control in Autoimmunity Sample Diluent from 1:1 to 1:32. Standards in the range from C:1 to C:32 are obtained.

Remove the required number of strips from the aluminium pouch sufficient for the number of assays to be performed. Place the strips in the frame provided. To the appropriate wells, add the reagents and perform the various assay steps as indicated on the following table:

Reagent	Volume	Procedure
Anti-β ₂ GPI IgG Positive Control dilutions, Negative Control, diluted test sample or Sample Diluent (blank)	200 µL	Add the Positive Control, Negative Control, or diluted test sample to the appropriate microwell
Incubate for 30 minutes at room temperature (18°-25°C)		
Wash Solution	300 µL	Wash the wells 5 times.
Anti-IgG (Fcγ)-HRP Immunconjugate	200 µL	Add the conjugate to each microwell immediately after the wash step
Incubate for 30 minutes at room temperature (18°-25°C)		
Wash Solution	300 µL	Wash the wells 5 times
TMB Substrate	200 µL	Add the substrate to each microwell immediately after the wash step
Incubate for exactly 5 minutes at room temperature (18-25°C)		
0.45M H ₂ SO ₄	50 µL	Following exactly the same time intervals used for adding the substrate, stop the reaction by adding 0.45M H ₂ SO ₄
Wait for 10 minutes in order to allow the color to stabilize and measure the absorbance at 450 nm. Subtract the blank value from the measurements.		

Notes:

1. Avoid letting the plate in the bright sunlight during incubations and particularly during color development.
2. Do not allow the microwells to dry out between the addition of reagents or following a washing step. Add the next reagent within 3 minutes in order to prevent the microwells from drying, which could damage the immobilized components. If necessary, fill the microwells with Wash Solution and empty it just before the introduction of the next reagent.
3. When adding the TMB substrate, the time interval between each row must be accurate and exactly determined. It must be the same when stopping the reaction.
4. For biochromatic readings, use a reference wavelength at 690 nm or at 620 nm.

CALIBRATION CURVE

Construct a standard curve by plotting the anti-β₂GPI concentrations expressed in AU on the x-axis and the corresponding A₄₅₀ on the y-axis. A standard curve should be generated each time the assay is performed.

CALCULATIONS

The anti-β₂GPI IgG concentration for the sample tested at the 1:100 dilution, expressed in AU, is deduced directly from the curve. If higher dilutions are used, the concentration measured must be multiplied by the complementary dilution factor (i.e. multiply the concentration by 2 for a 1:200 sample dilution or by 4 for a 1:400 sample dilution). Alternatively, an ELISA software (i.e. Dynex, etc.) may be used for the calculation of concentrations.

RESULTS

Normal Range

The Positive Control unit (AU) is defined as the upper limit of the normal range, which corresponds to the mean value obtained in a normal population plus 2 standard deviations (SD). By definition, this corresponds to 10 AU.

Normal Range: < 10 AU/mL

Grey Zone

A "grey zone" is defined because some pathological samples (inflammation, infectious diseases, autoimmune diseases, gammopathy, elderly people) can produce higher backgrounds in autoimmune assays than normal individuals that can mimic or mask a low reactivity. If a patient is in the grey zone, it is recommended to perform a new test on a fresh sample at a later time in order to follow a possible ongoing generation of autoantibodies to β₂GPI of the IgG isotype.

Grey Zone: ≥ 10 to < 20 AU/mL

Positive Range

The positive range is defined as anti-β₂GPI autoantibody concentrations ≥ 20 AU/mL. The positive range can be classified as follows:

Low Positive: ≥ 20 to < 50 AU/mL
Moderate Positive: ≥ 50 to < 100 AU/mL
High Positive: ≥ 100 AU/mL

QUALITY CONTROL

Positive and negative controls provided in the kit allow validating the assay. Expected A₄₅₀ values for positive and negative controls can present variations from lot to lot but they always are,

Positive Control A₄₅₀ ≥ 1.5 Negative Control A₄₅₀ ≤ 0.25

LIMITATIONS OF THE PROCEDURE

If the wash steps are not correctly performed, the negative control can produce a high absorbance value. In order to avoid non-specific color development, check that the wash steps are performed efficiently. As for any autoantibody assay, the presence of inflammation, infectious diseases, autoimmune diseases, immune-complexes and high concentrations of IgG in the sample may induce a high background that can be within the grey zone or in the low positive range.

EXPECTED VALUES

Autoantibodies to β₂GPI are usually absent in normal population. Their presence at moderate or high concentrations may be associated with recurrent abortions, miscarriages, the anti-phospholipid syndrome and thrombotic diseases. The pathological effect of autoantibodies to β₂GPI is still discussed, but these latter are thought to contribute to trigger hypercoagulability. Pathogenicity of the various isotypes is still not completely understood. The severity of clinical manifestations associated with the presence of autoantibodies to β₂GPI, increases with the IgG isotype, the antibody concentration and its affinity, and the time of exposure.

PERFORMANCE CHARACTERISTICS

The IMUCLONE® Anti-β₂GPI IgG ELISA specifically measures human autoantibodies to β₂GPI of the IgG isotype, reactive with immobilised β₂GPI. IgM or IgA isotypes are not measured.

REFERENCES

1. Arvieux, J., Roussel, B., Jacob, M. C. and Colomb, M.G. Measurement of antiphospholipid antibodies by ELISA using β₂-Glycoprotein I as antigen. *J. Immunol. Meth.* 1991, **143**: 223-229.
2. Viard, J. P., Amoura, Z. and Bach, J. F. Association of Anti β₂-Glycoprotein I Antibodies with Lupus Type Circulating Anticoagulant and Thrombosis in Systemic Lupus Erythematosus. *Am. J. Med.* 1992, **93**: 181-86.
3. Martinuzzo, M. E., Forastiero, R. P. and Carreras, L. O. Anti β₂-Glycoprotein I antibodies : detection and association with Thrombosis. *Brit. J. Haemat.* 1995, **89**: 397-402.
4. Sanmarco, M. and Soler, C. Heterogeneity of β₂-Glycoprotein I antibodies. *Nouv. Rev. Re. Haemat.* 1995, **37**: S57-S60.
5. Amengual, O., Atsumi, T., Khamashta, M. A. and Hughes, G. R. V. Clinical significance of anti β₂-Glycoprotein I antibodies. *Am. Med. Interne*, 1997, **147-1**: 15-17.