



ZYMUTEST ANTI β_2 -GLYCOPROTEIN I

IgG - Isotype

Ref: RK014A-RUO

Auto-antibodies to β_2 -Glycoprotein I (β_2 GPI), IgG isotype

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

English, last revision: 11-2022

INTENDED USE:

The ZYMUTEST anti- β_2 GPI, IgG ELISA kit, is an optimized enzyme immuno-assay designed for measuring auto-antibodies to β_2 GPI of the IgG isotype, in human plasma or serum or in any biological fluid where auto-antibodies to β_2 GPI must be measured.

This kit is for research use only and should not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

The ZYMUTEST anti- β_2 GPI, IgG Kit, specifically measures human auto and alloantibodies to β_2 GPI of the IgG isotype, reactive with immobilized β_2 GPI. IgM or IgA isotypes are not measured. This assay is designed with native uncleaved and non-altered, highly purified human β_2 GPI, which has then a preserved structure. This method then provides high reproducibility, high sensitivity and high specificity.

ASSAY PRINCIPLE:

Search of anti- β_2 GPI antibody, with ZYMUTEST anti- β_2 GPI kit, is performed using an ELISA plate, sensitized by the native human β_2 GPI then stabilized.

The diluted plasma or serum sample or biological fluid is introduced into one of the microwells of the β_2 GPI coated plate. When present, anti- β_2 GPI auto-antibodies bind to immobilized β_2 GPI. Following a washing step, bound auto-antibodies, of the IgG isotype, are revealed by an immunoconjugate, goat anti-human IgG (Fc γ specific)-peroxidase conjugate, which reacts specifically with IgG isotypes. Following a new washing step, the peroxidase substrate, 3,3',5,5'-Tetramethylbenzidine (TMB) in presence of hydrogen peroxide (H₂O₂), is introduced and a blue colour develops. The colour turns yellow when the reaction is stopped with sulfuric acid. The colour developed is directly proportional to the amount of anti- β_2 GPI auto-antibodies, of the IgG isotype, present in the tested sample.

Tested samples:

- Trisodium citrate or EDTA anticoagulated human plasma or human serum.
- Any biological fluid, where human auto-antibodies to β_2 GPI, of the IgG isotype, must be assayed.

REAGENTS:

- COAT: Micro ELISA plate:** containing 12 strips of 8 wells, coated with highly purified human β_2 GPI, then stabilized; the plate is packed in an aluminium pouch hermetically sealed in presence of a desiccant.
- SD: Autoimmunity Sample Diluent:** 2 vials containing 50 mL of Autoimmunity Sample Diluent, ready to use.
- CAL: Anti- β_2 GPI IgG Calibrator:** 3 vials of calibrator, lyophilized. After reconstitution with 1 mL of Autoimmunity Sample Diluent, the calibrator is ready to use (already diluted 1:100).
- C-: Negative Control:** 3 vials of negative control, lyophilized containing diluted normal human plasma. After reconstitution with 1 mL of Autoimmunity Sample Diluent, the negative control is ready to use (already diluted 1:100).
- IC: Anti-IgG-Peroxidase:** 3 vials of immunoconjugate (Anti-IgG (Fc γ)-HRP immunoconjugate), goat polyclonal antibodies specific for human IgG-Fc γ coupled to HRP, lyophilized.
- CD: Conjugate Diluent:** 1 vial of 25 mL of immunoconjugate diluent, ready to use.
- WS: Wash Solution:** 1 vial of 50 mL of 20 fold concentrated Wash Solution.
- TMB: Tetramethylbenzidine:** 1 vial of 25 mL peroxidase substrate (3,3',5,5' - Tetramethylbenzidine) containing hydrogen peroxide, ready to use.
- SA: Stop Solution:** 1 vial of 6 mL of 0.45M Sulfuric acid, ready to use.

The exact concentration of calibrator and control is indicated on the flyer provided in the kit. The anti- β_2 GPI concentrations for the calibrators, expressed in Arbitrary Units (AU), may slightly vary from lot to lot. For the assay, refer to the concentration indicated on the flyer provided in the kit used.

CAUTIONS AND WARNINGS:

- Any product of biological origin must then be handled carefully, as being potentially infectious.
- If the substrate becomes yellow, this indicates the presence of a contaminant. It must be rejected, and a new vial must be used.
- The disposal of waste materials must be carried out according to current local regulations.
- Use only reagents from kits with the same lot number. Do not mix reagents from kits with different lots when running the assay; they are optimized for each lot of kits.
- Reagents must be handled with care, in order to avoid any contamination during use. Take care to limit as much as possible any evaporation of the reagents during use, by limiting the liquid-air surface exchange. Evaporation reduces reagent stability on instrument board.
- In order to preserve the stability of the reagents, close the vials with their original screw cap following each use.
- Stability studies for 3 weeks at 30°C show that the reagents can be shipped at room temperature for a short period without damage.
- For in vitro use.

PREPARATION AND STABILITY OF REAGENTS:

Bring the kit at room temperature, at least 30 min before use. Store the unused reagents at 2-8°C. Vials are closed under vacuum. Remove carefully the stopper, in order to avoid any loss of powder when opening the vials.

- COAT:** Open the aluminium pouch and take off the required amounts of 8 well strips for the test series. When out of the pouch, the strips must be used within 30 minutes. Unused strips can be stored at 2-8°C for 4 weeks in their original aluminium pouch, in presence of the desiccant, hermetically closed and protected from any moisture, and stored in the provided plastic microplate storage bag (minigrip).

- SD:** Ready to use. Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
 - 4 weeks at 2-8°C
- CAL:** Reconstitute each vial with 1 mL of Autoimmunity Sample Diluent, shake thoroughly for complete dissolution. The obtained calibrator is ready to use calibrator. It corresponds to a plasma containing IgG isotype auto-antibodies to β_2 GPI, already diluted 1:100.
 - Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
 - 5 days at 2-8°C
- C-:** Reconstitute each vial with 1 mL of Autoimmunity Sample Diluent, shake thoroughly for complete dissolution. The obtained negative control is ready to use. It corresponds to a normal human plasma, already diluted 1:100.
 - Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
 - 2 weeks at 2-8°C
- IC:** Reconstitute each vial with 7.5 mL of Conjugate Diluent at least 15 min before use. Let the pellet to be completely dissolved before use, and shake the vial gently in order to homogenize the content.
 - Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
 - 4 weeks at 2-8°C
 - 24 hours at room temperature (18-25°C)
- CD:** Ready to use. This reagent contains 0.05% Kathon CG. Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
 - 4 weeks at 2-8°C
- WS:** Incubate, if necessary, the vial in a water bath, at 37°C, until complete dissolution of crystals. Shake the vial and dilute the amount required 1:20 in distilled water (the 50 mL contained in the vial allow to prepare 1 liter of Wash Solution).
 - Stability of the wash solution, provided that any contamination or evaporation is avoided, kept in its original vial:
 - 4 weeks at 2-8°C
- TMB:** Ready to use. Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
 - 4 weeks at 2-8°C
- SA:** Stop solution containing 0.45M sulfuric acid, ready to use.

STORAGE CONDITIONS:

Unopened reagents must be stored at 2-8°C, in their original packaging box. They are usable until the expiration date printed on the label.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water.

Materials:

- 8-channel or repeating pipette allowing dispensing volumes of 50-300 μ L.
- 1-channel pipettes at variable volumes from 0 to 20 μ L, 20 to 200 μ L and 200 to 1000 μ L.
- Micro ELISA plate washing equipment and shaker.
- Micro ELISA plate reader with a wavelength set up at 450 nm.

SPECIMEN COLLECTION:

Preparation and storage of specimens must be performed according to the current local regulations.

Specimens:

Human plasma obtained from trisodium citrate anticoagulated blood. EDTA collected human plasma may also be used. The storage conditions are the same with citrated plasma. Auto-antibodies to β_2 GPI can also be assayed on serum. However it is better to perform the assays on plasma.

Collection:

Blood (9 vol.) must be collected on trisodium citrate anticoagulant (1 vol.) (0.109M), with caution, through a net venipuncture. The first tube must be discarded.

Centrifugation:

Within 2 hours, use a validated method in the laboratory to obtain a platelet-poor plasma, e.g., a minimum of 15 minutes at 2500 g at room temperature (18-25°C) and plasma must be decanted into a plastic tube.

Storage of plasma:

- 8 hours at room temperature (18-25°C)
- 48 hours at 2-8°C.
- 1 month at -20°C.

Frozen plasma specimens should be rapidly thawed at 37°C, then gently mixed and tested immediately. Resuspend any precipitation by thorough mixing immediately after thawing and before testing.

TEST PROCEDURE:

Assay procedure:

- Calibrator and negative control are ready to use (already diluted 1:100).

- The samples should be diluted using SD solution as described in the table below:

Samples	Dilution
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Plasma	1:100
Serum	1:100
Biological fluid	1/100

When high amounts of auto-antibodies to β_2 GPI are expected, dilute at **1:200** or **1:400** dilutions. Results must then be multiplied by **2** or **4**.

3. Remove the required number of strips from the aluminium pouch and put the strips in the frame provided. In the different wells of the micro ELISA plate, introduce the reagents and perform the various assay steps as indicated on the following table:

Reagent	Volume	Procedure
Anti- β_2 GPI IgG Calibrator or Negative control or 1:100 diluted sample or Sample diluent (blank)	200 μ L	Introduce the : – Calibrator or – negative control or – diluted sample or – sample diluent into the micro ELISA plate wells.
Incubate for 30 minutes at room temperature (18-25 °C) (a) (b)		
Wash Solution (20 fold diluted in distilled water)	300 μ L	Proceed to 5 successive washings. (b)
Conjugate anti-IgG (Fc γ)-HRP immunoconjugate, restored with 7.5 mL of conjugate diluent	200 μ L	Immediately after the washing, Introduce the anti-IgG (Fc γ)-HRP immunoconjugate in the micro ELISA plate wells.
Incubate for 30 minutes at room temperature (18-25 °C) (a)		
Wash Solution (20 fold diluted in distilled water)	300 μ L	Proceed to 5 successive washings (b)
TMB/H ₂ O ₂ Substrate	200 μ L	Immediately after the washing, introduce the substrate into the wells. Nota: The substrate distribution, row by row, must be accurate and at exact time intervals (c) .
Let the colour develop for 5 min. at room temperature (18-25 °C) (a)		
0.45M Sulfuric Acid	50 μ L	Following exactly the same time intervals than for the addition of substrate, stop the colour development by introducing the 0.45M sulfuric acid (c)
Wait for 10 minutes in order to allow the colour to stabilize and measure absorbance at 450 nm. Subtract the blank value (d).		

Note:

- a) Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro ELISA plate shaker can be used.
- b) Never let the plates empty between the addition of the reagents or following the washing step to preserve the immobilized proteins. The next reagent must be added within 3 minutes, in order to prevent the plate from drying, which could damage the immobilized proteins and reduce plate reactivity. If necessary, keep the plate filled with Wash Solution and empty it just before the introduction of the next reagent. The washing instrument must be adjusted in order to wash the plates gently, and to avoid a too drastic emptying, which could lower plate reactivity.
- c) For addition of the TMB substrate, the time interval between each row must be accurate and exactly determined. It must be the same when stopping the reaction by sulfuric acid.
- d) For a bichromatic reading, the reference wavelength at 690 nm or at 620 nm can be used.

VALIDATION:

- Calibrator and control provided in the kit allow validating the right performance of the assay.
- Expected OD values for calibrator (CAL) and the negative control (C-) can present variations from lot to lot but, when the assay is performed at room temperature, between 18 and 25°C, they always are:

OD₄₅₀ for 1:1 calibrator : ≥ 1.5

OD₄₅₀ for negative control : ≤ 0.25

Concentrations obtained for calibrator and negative control, at 18-25°C, are indicated on the flyer provided in the kit for each reagent lot.

QUALITY CONTROL:

Using quality controls, allows validating the calibration curve, as well as the homogeneous reactivity from run to run, when using a same lot of reagents.

Quality control must be included in each series, as per good laboratory practice, in order to validate test results. A new calibration curve must be carried out preferentially for each test series, and at least for each new lot of reagents or, after each important analyzer's maintenance, or when quality controls values are measured outside the acceptance range determined for the method.

Each laboratory should establish and verify its own target values, acceptance ranges and expected performances, according to the instruments and protocols used.

RESULTS:

- Results are expressed according to the **OD₄₅₀** values obtained for samples and control using the calibration curve.
- For the manual method, draw the calibration curve on a bi-logarithmic graph paper plot, with on abscissae the anti- β_2 GPI concentration (**AU**) and on ordinates the corresponding **OD₄₅₀**. The anti- β_2 GPI, autoantibody concentration, of the IgG isotype, for the sample, tested at the standard **1:100** dilution, and expressed in **AU**, is directly deduced from the calibration curve.
- When higher dilutions are used, (i.e. **D**), the concentration measured must be multiplied by the complementary dilution factor (i.e. **D:100** ; for example **x2** for **1:200** or **x4** for **1:400**).
- Alternatively, specific software (i.e. Dynex, Biolise, etc...), can be used for the calculation of concentrations.

The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

LIMITATIONS:

- In order to get the optimal performances of the assay, the technical instructions must be strictly respected.
- Any reagent presenting an unusual aspect or contamination signs must be rejected.
- Any plasma containing a coagulum or contamination signs must be rejected.
- If washing steps are not correctly performed, it can induce high background and a high absorbance value of the negative control. In order to avoid non-specific colour development, check that the washing step is efficiently and correctly performed.

SYMBOLS:

Used symbols and signs listed in the ISO standard 15223-1.

Changes compared to the previous version.