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ZYMUTEST β2GPI

 # RK022A

(Complete ELISA kit for measuring human β2GPI Antigen)

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTENDED USE:

The ZYMUTEST β2GPI kit is an immuno-assay for measuring human Beta 2 Glyocoprotein I (β2GPI) in plasma, or in any fluid where β2GPI can be present.
This kit is for research use only and should not be used for patient diagnosis or treatment.

ASSAY PRINCIPLE:

The ZYMUTEST β2GP1 is a one step enzyme immuno-assay for the measurement of β2GP1 in plasma or biological milieu. First, the immunoconjugate, a goat polyclonal antibody, specific for human β2GP1, and coupled to horse-radish-peroxidase (HRP), is introduced into the microwell, coated with a polyclonal antibody specific for β2GP1. Then, the diluted test sample is introduced and the immunological reaction starts. When present, β2GP1 binds to the coated microwell and reacts with the immunoconjugate, simultaneously. Following a washing step, the peroxidase substrate, tetramethylbenzidine (TMB) in presence of hydrogen peroxide (H_2O_2), is introduced and a blue colour develops. The colour turns yellow when the reaction is stopped with Sulfuric Acid. The amount of colour developed is directly proportional to the concentration of β2GP1 in the tested sample.

TEST SAMPLE:

- Trisodium Citrate or Na_2 EDTA anticoagulated human plasma.
- The assay can be performed on serum.
- Any biological fluid where β2GPI:Ag must be measured.

REAGENTS:

1. **COAT: Micro ELISA plate,** containing 12 strips of 8 wells, coated with highly purified polyclonal antibodies specific for human β2GPI, then stabilised; the plate is packed in an aluminium pouch hermetically sealed in presence of a desiccant.
2. **SD:** 2 vials containing 60ml of **B2F-Sample Diluent**, ready to use.
3. **STD:** 3 vials of **β2GPI Calibrator**, lyophilised. When restored with **2 ml** of B2F-Sample Diluent, a solution containing **"C" ng/ml** of human β2GPI is obtained.
4. **CI:** 1 vial containing **1 ml** of lyophilised **(h) β2GPI Control I** (**High**).
5. **CII**: 1 vial containing **1 ml** of lyophilised **(h) β2GPI Control II (Low)**.

> ***Note:*** The β2GPI concentrations and acceptancy ranges for calibrator and controls can vary from lot to lot, but are precisely indicated for each lot on the flyer provided in the kit.

6. **IC:** 3 vials of **Anti-(h)-β2GPI-HRP immunoconjugate**, a polyclonal antibody coupled to HRP, lyophilised.
7. **CD**: 1 vial of 25 ml of **B2F-Conjugate Diluent**, ready to use.
8. **WS:** 1 vial of 50 ml of 20 fold concentrated **Wash Solution**.
9. **TMB:** 1 vial of 25 ml peroxidase substrate: **3,3',5,5' – Tetramethylbenzidine** containing hydrogen peroxide. Ready to use.
10. **SA:** 1 vial of 6 ml of **0.45M Sulfuric acid (Stop solution)**. Ready to use.

> ***Note:*** *Use only components from a same kit lot. Do not mix components from different lots of kits, when running the assay.*

REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED:

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

REAGENTS PREPARATION, STORAGE AND STABILITY:

In their original packaging box, before use, when stored at 2-8°C, the unopened reagents are stable until the expiration date printed on the box.

1. **Micro ELISA plate:** open the plastic 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 microplate storage bag (minigrip).
2. **B2F-Sample Diluent:** It is ready to use. When open, it can be used for **4 weeks**, stored at **2-8 °C**, and provided that any bacterial contamination is avoided during use. This reagent contains 0.05% Kathon CG.
3. **β2GPI Calibrator:** restore each vial with **2 ml** of B2F-Sample Diluent in order to obtain a solution containing **"C" ng/ml** of β2GPI. This solution is stable for at least **8 hours** at room temperature (18-25°C) or **72 hours at 2-8°C.**
4. **β2GPI Control I** (high): restore with **1 ml** of B2F-Sample Diluent.
5. **β2GPI Control II** (low): restore with **1 ml** of B2F-Sample Diluent.

> ***Note:*** when restored, β2GPI controls are stable for **8 hours** at **room temperature**, **72 hours at 2-8°C** or **2 months** frozen **at –20°C** or below.
>
> ***Warning***: Purified (h) β2GPI used for the preparation of calibrator (3) and controls (4&5) is prepared with normal human plasma. This latter was tested with registered methods and found negative for HIV antibodies, HBs Ag and HVC antibodies. However, no assay may warrant the total absence of infectious agents. Any product of human origin must then be handled with all the required cautions, as being potentially infectious.

6. **Anti-(h)-β2GPI-HRP immunoconjugate**: each vial must be restored with **7.5 ml of B2F-Conjugate Diluent**. Let the pellet completely dissolve before use, and shake the vial gently in order to homogenize the contents. The restored conjugate is stable for at least **24 hours** at room temperature or for at least **4 weeks at 2-8°C**.
7. **B2F-Conjugate Diluent**: It is ready to use. When open, it can be used for **4 weeks**, stored at **2-8 °C**, and provided that any bacterial contamination is avoided during use. This reagent contains 0.05% Kathon CG.
8. **Wash Solution**: Incubate the vial for 15-30 minutes in a water bath **at 37°C** until complete dissolution of solids, when present. Shake the vial and dilute the amount required 1:20 in distilled water (the 50 ml contained in the vial allow preparing 1 liter of Wash Solution). The Wash Solution must be stored at **2-8°C** in its original vial and used within **4 weeks** following opening. The diluted Wash Solution must be used within **7 days**, when protected from any contamination and stored at **2-8°C**. This reagent contains 0.05% Kathon CG.
9. **TMB substrate:** It is ready to use. When open, it can be used for **4 weeks**, stored at **2-8°C**, and provided that any bacterial contamination is avoided during use.
10. **Stop solution:** It is ready to use.

> ***Note*:** Bring the kit at room temperature, at least 30 min. before use. Store the unused reagents at 2-8°C.
> The stability studies at 30°C show that the reagents can be shipped at room temperature without damage.

PROCEDURE:

Specimen collection:
Blood (9 vol.) must be collected on 0.109M citrate anticoagulant (1 vol.) by a clean venipuncture; plasma supernatant is decanted following a 20 min. centrifugation at 2,500 g; citrated plasma should be tested **within 8 hours** or stored frozen at –20°C or below for up to 6 months, and thawed for 15 min. at 37°C just before use. Thawed specimen must be tested within **4 hours**.
EDTA collected human plasma may also be used. Conditions of storage are the same than those for citrated plasma.

Tested plasma or sample or controls:

- The sample must be tested diluted in order to obtain a concentration **<100 ng/ml** in the B2F-Sample Diluent.

For example, a human plasma must be tested at the **1:4,000** dilution in the B2F-Sample Diluent (SD).

For an improved accuracy, when high dilutions are required, proceed by successive dilutions as follows:
Add 20 µl of plasma into 180µl of B2F-Sample Diluent (D=1:10); mix for homogenizing.
Then add 20 µl of this "1:10 dilution" into 180 µl of B2F-Sample Diluent (D=1:10); mix for homogenizing (D=1:100 final).
Then add 20 µl of this "1:100 dilution" into 780 µl of B2F-Sample Diluent (D=1:40); mix for homogenizing. It allows obtaining a final dilution of **1:4,000** for the assayed sample

Controls I & II, restored with **1 ml** of B2F-Sample Diluent (SD), are already diluted 1:4,000 before lyophilisation and must then be tested "undiluted".

- For tested specimen different from plasma, adjust the assayed dilution according to the expected β2GPI concentration, in order to have this latter within the dynamic range, i.e., 5 to 100 ng/ml. In order to avoid any matrix effect, use at least a 1:5 dilution in the B2F-sample diluent (SD)

Calibration:
Using the **"C" ng/ml** β2GPI calibrator provided in the kit, prepare the following standard solutions.

β2GPI concentration (ng/ml)	C	C:2	C:4	C:10	C:20	0
Vol. of β2GPI calibrator	1 ml	0.5 ml	0.25 ml	0.1 ml	0.05 ml	0 ml
Vol. of B2F-Sample Diluent	0 ml	0.5 ml	0.75 ml	0.9 ml	0.95 ml	1 ml

Mix gently for a complete homogenization.
The standard dilutions are stable for at least **4 hours** at room temperature.

Assay procedure:
Remove the required number of strips from the aluminium pouch, for the series of measures to be performed. Then 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
Conjugate (anti B2GPI antibody coupled with peroxidase; restored with 7.5 ml of B2F-Conjugate Diluent)	**200 µl**	Introduce the Anti-(h)-B2GPI- HRP immunoconjugate in the micro ELISA plate wells
β2GPI standards (1:1), or tested sample diluted 1:4,000, or controls (1:1), or B2F-Sample Diluent (blank)	**50 µl**	Introduce the standard solutions or the tested samples in the corresponding micro ELISA plate well (a).
Incubate for 1 hour at room temperature (18-25°C) (b)		
Wash Solution (20 fold diluted in distilled water)	**300 µl**	Proceed to 5 successive washings using the washing instrument **(b).**
TMB/H_2O_2 Substrate	**200 µl**	Immediately after the washing, introduce the substrate into the wells. **Note:** The substrate distribution, row by row, must be accurate and at exact time intervals **(c, d)**.
Incubate for exactly 5 minutes at room temperature (18-25 °C) (b)		
0.45M Sulfuric Acid	**50 µl**	Following exactly the same time intervals than for the addition of substrate, stop the colour development by introducing the 0.45M sulfuric acid**.**
Wait for **10 minutes** in order to allow the colour to stabilize and measure absorbance at **450 nm (A450) (e)**. Subtract the blank values		

Note:

a) Distribute calibrators, controls and tested specimen as rapidly as possible (within 10 minutes), in order to obtain homogeneous immunological kinetics for β2GPI binding.

b) Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro-ELISA plate shaker can be used.

c) Never let the plates empty between the addition of the reagents or following the washing step. The next reagent must be added within 3 minutes, in order to prevent the plate from drying, which could damage the immobilized components. 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.

d) 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.

e) For bichromatic readings, a reference wavelength at 690 nm or at 620 nm can be used.

EXPRESSION OF RESULTS:

- On a linear graph paper plot the β2GPI:Ag concentration, in **ng/ml**, on abscissa and the corresponding absorbance (**A450**) on ordinates (See model on the flyer).
- Users must construct their own calibration curve, obtained using their standard dilutions. From the curve obtained, deduce the β2GPI:Ag concentration for the diluted tested sample. For obtaining the β2GPI concentration in this undiluted sample, this value must be **multiplied by the dilution factor used** (e.g., **4,000**…).
- For **controls I and II**, the concentrations measured are directly deduced from the curve.
- Alternatively, an ELISA 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.

BIOCHEMISTRY:

β2GPI (Beta 2- glycoprotein I, also called apolipoprotein H) is a 50 kDa plasma glycoprotein, with a high affinity for negatively charged phospholipid surfaces.
Its physiological role remains unclear; however β2GPI shows a variety of anticoagulant properties in vitro, by interfering with the anionic phospholipid surfaces onto coagulation processes are focused. The expected β2GPI:Ag concentration in normal human plasma is about 200 µg/ml (about 180 ± 100µg/ml when determined with Zymutest β2GPI kit).

ASSAY CHARACTERISTICS:

- Detection threshold ≤5 ng/ml (directly read on the curve).
- Intra-assay CV: 3-8%.
- Inter-assay CV: 5-10%.
- Reference material: The β2GPI calibrator of the ZYMUTEST β2GPI kit is established against a normal citrated plasma pool, and against highly purified human β2GPI, which protein concentration has been precisely determined by Lowry.

Changes compared to the previous version.