USER GUIDE

Human anti-β2GP1 ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human anti-β2GP1

Catalog Numbers ENDO-APS

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **endotelix.com/support**.

Product description

The Human anti-β2GP1 ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human anti-β2GP1

For Research Use Only. Not for use in diagnostic procedures.

Reagents provided

Reagents for Human ENDO-APS ELISA Kit (96 tests)

1 aluminum pouches with a Microwell Plate (12 strips with 8 well each) coated with custom Protein.

2 vials (2 x 50 ml) Wash Buffer
(**WARNING:** pH should be adjust at 7.2)

1 vials (12 mL) Detection Solution

1 vials (12 mL) TMB Substrate Solution

1 vials (6 mL) Stop Solution

1 vials human calibrator antibody

Storage instructions – ELISA kit

The kit is send at room temperature but should be store between 2° and 8°C.

Sample collection and storage instructions

Cell culture supernatant, serum and plasma (citrate and heparin) were tested with this assay. Other biological samples might be suitable for use in the assay.

Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Materials required but not provided

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

Precautions for use

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipet by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.

STORAGE CONDITIONS

• Microplate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 6 month at 2-8 °C.*
• Wash Buffer (5x)	May be stored for up to 6 month at 16-25 °C.
• Calibrator antibody	Aliquot and store for up to 6 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze thaw cycles. May be stored for up to 1 month at 2-8 °C.*
• Detection Solution	May be stored for up to 2 month at 2-8 °C.*
• TMB Substrate Solution	May be stored for up to 6 month at 2-8 °C.*
• Stop Solution	May be stored for up to 6 month at 2-8 °C.*

* Provided this is within the expiration date of the kit.

Preparation of reagents

1. Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure.
2. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

Wash buffer pH 7.2 (1x)

1. Pour entire contents (2x50 mL) of the Wash Buffer Concentrate (5x) into a clean 500 mL graduated cylinder. Bring to final volume of 500 mL with glass-distilled or deionized water.
2. Mix gently to avoid foaming.
3. Check and Adjust the pH at 7.2 with HCl 37%
4. Transfer to a clean wash bottle and store at 16° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.
5. Wash Buffer (1x) may also be prepared as needed.

Samples

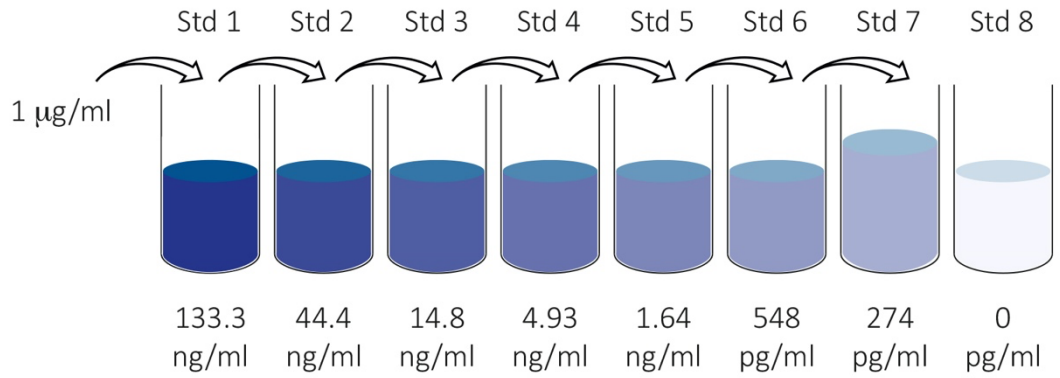
For Human samples: Serum or plasma should be dilute in PBS between 1/1'000 to1/10'000 (Classical dilution is 1/1'000)

For cell supernatant: Should be determined

Standard Curve

The ready to use stock solution of 1 µg/mL.

Pipette 520 µL of Phosphate Buffer Saline pH 7.2 into the Std 1 tube (133.3 ng/mL). Pipette 300 µL of Phosphate Buffer Saline pH 7.2 into the remaining tubes (Std 2 to Std 8). Put 80 µL of the stock solution into the Std 1 tube to produce a dilution series (below). For dilution series, transfer 150 µL to next tube (Std 2 to Std 7). Mix each tube thoroughly before the next transfer. The Std 1 tube (133.3 ng/mL) standard serves as the high standard. The Phosphate Buffer Saline pH 7.2 serves as the zero standard Std 8 tube (0 pg/mL).



Test protocol summary

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. Add 100 µL of standard, control, or sample per well in duplicate with right dilution.
4. Cover microwell strips and incubate 1.5 hours at room temperature with gentle agitation (18° to 25°C)
5. Empty and wash microwell strips 3 times with Wash Buffer.
6. Add 100 µL Detection Solution to all wells.
7. Cover microwell strips and incubate 0.5 hour at room temperature with gentle agitation (18º to 25ºC).
8. Empty and wash microwell strips 3 times with Wash Buffer.
9. Add 100 µL of TMB Substrate Solution to all wells.
10. Incubate the microwell strips for about 1 to 5 minutes at room temperature (18°to 25°C).
11. Add 50 µL Stop Solution to all wells.
12. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.