ITGA2B/ITGB3 (Human) ELISA Kit

Catalog Number KA0477

96 assays

Version: 03

Intended for research use only

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Introduction

Background

Platelet membrane glycoprotein IIb/IIIa (GPIIb/IIIa, integrin $\alpha_{IIb}\beta_3$) is a member of the integrin family of cell membrane receptors that play key roles in thrombus formation, platelet aggregation, embryogenesis, and intercellular adhesion. Each integrin receptor complex consists of a heavy (alpha) and a light (beta) chain associated as a calcium-dependent heterodimer with a molecular mass of 140 kDa and 90 kDa respectively (1). GPIIb/IIIa serves as an inducible receptor for fibrinogen, fibronectin, von Willebrand factor, and vitronectin (2, 3). The simultaneous occupancy on adjacent platelets of receptors with dimeric fibrinogen molecules leads to platelet aggregation. Hereditary defects of the GPIIb/IIIa receptor cause Glanzmann's thrombasthenia (GT), an autosomal recessive bleeding disorder (4).

Principle of the Assay

The ITGA2B/ITGB3 (Human) ELISA Kit is designed for detection of human GPIIb/IIIa in platelets, platelet-rich plasma, and cell culture lysate samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures GPIIb/IIIa in less than 4 hours. A polyclonal antibody specific for human GPIIb/IIIa has been pre-coated onto a 96-well microplate with removable strips. GPIIb/IIIa in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for GPIIb/IIIa, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

General Information

Materials Supplied

List of component

Component	Amount
Human GPIIb/IIIa Microplate: A 96-well polystyrene microplate coated with a polyclonal antibody against GPIIb/IIIa.	96 (8x12) wells
Sealing Tapes: Pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Human GPIIb/IIIa Standard: Human platelet GPIIb/IIIa in a buffered protein base (lyophilized).	60 ng
Biotinylated Human GPIIb/IIIa Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against human GPIIb/IIIa.	80 µl
MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base.	30 ml
Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant.	30 ml x 2
Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate.	80 µl
Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine.	8 ml
Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction.	12 ml

Storage Instruction

- ✓ Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated Antibody at -20°C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- ✓ Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- ✓ Diluent (1x) may be stored for up to 30 days at 2-8°C.
- ✓ Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel).

- ✓ Deionized or distilled reagent grade water.

Precautions for Use

- ✓ Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- ✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- ✓ This kit is for research use only.
- ✓ The kit should not be used beyond the expiration date.
- ✓ The Stop Solution is an acidic solution.

Assay Protocol

Reagent Preparation

- ✓ Freshly dilute all reagents and bring all reagents to room temperature before use.
- ✓ MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- ✓ Standard Curve: Reconstitute the 60 ng of Human GPIIb/IIIa Standard with 1.5 ml of MIX Diluent to generate a 40 ng/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (40 ng/ml) twofold with equal volume of MIX Diluent to produce 20, 10, 5, 2.5, and 1.25 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 3 days.

Standard Point	Dilution	[GPIIb/IIIa] (ng/ml)
P1	Standanrd (40 ng/ml)	40.0
P2	1 part P1 + 1 part MIX Diluent	20.0
P3	1 part P2 + 1 part MIX Diluent	10.0
P4	1 part P3 + 1 part MIX Diluent	5.00
P5	1 part P4 + 1 part MIX Diluent	2.50
P6	1 part P5 + 1 part MIX Diluent	1.25
P7	MIX Diluent	0.000

- ✓ Biotinylated Human GPIIb/IIIa Antibody (100x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- ✓ Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- ✓ SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Sample Preparation

- ✓ Platelet: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant containing 1 µM prostaglandin E1. Centrifuge samples at 100 x g for 15 minutes to obtain platelet-rich plasma. To sediment the platelets, the platelet-rich plasma is further centrifuged at 1000 x g for 10 minutes. The platelet pellet is then washed twice in Tyrode's HEPES buffer (pH 7.4) containing albumin (0.5%) and prostaglandin E1 (1 µM). The platelet is dissolved with 100 mM n-octylglycoside buffer (pH 7.4) in 20 mM

20 mM HEPES-buffered saline. Dilute samples 1:40 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- ✓ Platelet-Rich Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant containing 1 µM prostaglandin E1. Centrifuge samples at 100 x g for 15 minutes to obtain platelet-rich plasma. Dilute samples 1:40 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ Cell Culture Lysates: The cultured cells are lysed and solubilized with 15 mM octyl-β-D-glucopyranoside at 37°C for 15 minutes. Collect fresh cell lysates; dilute it with MIX Diluent and assay.

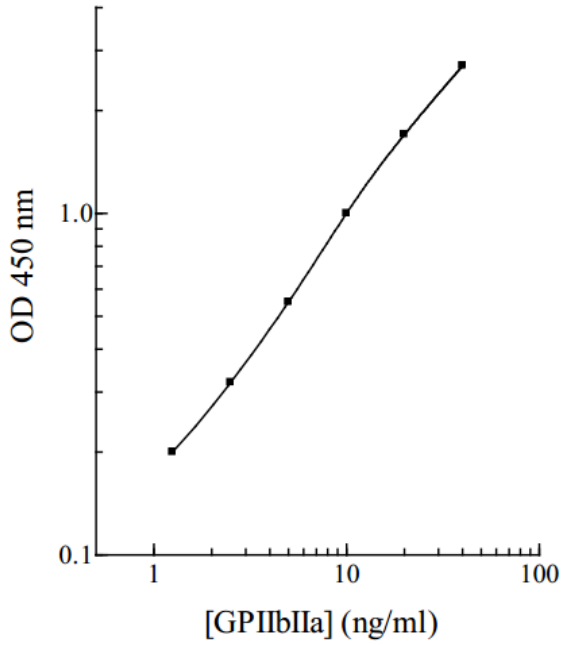
Assay Procedure

1. Prepare all reagents, working standards, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µl of Human GPIIb/IIIa Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
4. Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
5. Add 50 µl of Biotinylated Human GPIIb/IIIa Antibody to each well and incubate for 1 hour.
6. Wash the microplate as described above.
7. Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50 µl of Chromogen Substrate per well and incubate for about 12 minutes or till the optimal color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
10. Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
11. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections.
12. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings

Data Analysis

Calculation of Results

- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- ✓ Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.



The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

Performance Characteristics

- ✓ The minimum detectable dose of GPIIb/IIIa is typically ~ 1 ng/ml.
- ✓ Intra-assay and inter-assay coefficients of variation were 4.9% and 7.6% respectively.

- ✓ Linearity

	Average Percentage of Expected Value
Sample Dilution	Plasma
1:20	91%
1:40	98%
1:80	96%

✓ Recovery

Standard Added Value	2.5 - 20 ng/ml
Recovery %	86 - 113%
Average Recovery %	99%

✓ Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Monkey	20%
Mouse	2%
Rat	None
Swine	2%
Bovine	None
Rabbit	None

Resources

References

1. Kuhn, K. and Eble, J. (1994) Trends Cell Biol. 4:256
2. Kieffer, N. and Phillips, D.R. (1990) Annu. Rev. Cell Biol. 6:329
3. Ruggeri, Z.M. et al. (1983) J. Clin. Invest. 72:1
4. George, J. N. et al. (1990) Blood 75:1383

Plate Layout

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