Datasheet for ABIN612702

GPIIb/IIIa ELISA Kit

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Overview

Quantity:	96 tests
Target:	GPIIb/IIIa
Reactivity:	Human
Method Type:	Sandwich ELISA
Minimum Detection Limit:	1 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Human GPIIb/IIIa ELISA kit is designed for detection of human GPIIb/IIIa in platelets, platelet-rich plasma and cell culture lysate
Brand:	AssayMax
Sample Type:	Plasma, Blood
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	GPIIb/IIIa Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against GPIIb/IIIa. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay. 1 GPIIb/IIIa Standard: Human platelet GPIIb/IIIa in a buffered protein base (40 ng, lyophilized). Biotinylated GPIIb/IIIa Antibody (80x): A 80-fold concentrated biotinylated polyclonal antibody against human GPIIb/IIIa (120µl). EIA Diluent (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml).

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Product Details

	Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (90µl). 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).
Material not included:	Microplate reader capable of measuring absorbance at 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000µLand multiple channel). Deionized or distilled reagent grade water.

Target Details

Target:	GPIIb/IIIa
Alternative Name:	Glycoprotein IIb/IIIa (GPIIb/IIIa) (GPIIb/IIIa Products)
Background:	Platelet membrane glycoprotein IIb/IIIa (GPIIb/IIIa, integrin IIb 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 () and a light () chain associated as a calcium-dependent heterodimer with a molecular mass of 140 kDa and 90 kDa respectively. GPIIb/IIIa serves as an inducible receptor for fibrinogen, fibronectin, von Willebrand factor, and vitronectin. 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.

Application Details

Sample Volume:	50 µL
Assay Time:	< 4 h
Plate:	Pre-coated
Protocol:	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.
Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. 2 EIA

	Diluent (10x): Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 1 month at 2-8°C. Standard Curve: Reconstitute the 40 ng of human GPIIb/IIIa Standard with 1 ml of EIA Diluent to generate a 40 ng/ml of stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the GPIIb/IIIa standard solution (40 ng/ml) twofold with equal volume of EIA Diluent to produce 20, 10, 5.0, 2.50 and 1.25 ng/ml. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. [GPIIbIIIa] (ng/ml) Standard Point Dilution P1 1 part Standard (40 ng/ml) 40.000 P2 1 part P1 + 1 part EIA Diluent 20.000 P3 1 part P2 + 1 part EIA Diluent 10.000 P4 1 part P3 + 1 part EIA Diluent 5.000 P5 1 part P4 + 1 part EIA Diluent 2.500 P6 1 part P5 + 1 part EIA Diluent 1.250 P7 EIA Diluent 0.000 Biotinylated GPIIb/IIIa Antibody (80x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:80 with EIA Diluent. Any remaining solution should be frozen at -20°C. Wash Buffer Concentrate (20x): 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 EIA Diluent. Any remaining solution should be frozen at -20°C.
Sample Collection:	Platelet: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant containing 1 M 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 M). The platelet is dissolved with 100 mM n-octylglycoside buffer (pH 7.4) in 20 mM HEPES-buffered saline. Dilute samples 1:200 into EIA Diluent initially 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 M prostaglandin E1. Centrifuge samples at 100 x g for 15 minutes to obtain platelet-rich plasma. Dilute samples 1:200 into EIA 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--D- glucopyranoside at 370C for 15 minutes. Collect fresh cell lysates, dilute 1:2 with EIA Diluent and assay.
Assay Procedure:	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 - 30 °C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 µL of Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition. Wash five times

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Application Details

	with 200 µL of Wash Buffer. Invert the plate and decant the contents, and hit the plate 4-5 times on absorbent paper towel to complete remove liquid at each step. Add 50 µL of Biotinylated GPIIb/IIIa Antibody to each well and incubate for 30 minutes. Wash five times with 200 µL of Wash Buffer as above. 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. Wash five times with 200 µL of Wash Buffer as above. Add 50 µL of Chromogen Substrate per well and incubate for about 15 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. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. 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.
Calculation of Results:	Calculate the mean value of the duplicate or triplicate readings for each standard and sample. To generate a Standard Curve, plot 4-parameter 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. Standard Curve The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.
Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.7 % and 7.7% respectively.
Restrictions:	For Research Use only

Handling

Handling Advice:	The kit should not be used beyond the expiration date.
Storage:	4 °C/-20 °C
Storage Comment:	Store kit at 2-8°C or -20°C upon arrival up to the expiration date. Opened EIA Diluent may be stored for up to 1 month at 2-8°C. Store reconstituted reagents at -20°C or below. Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Publications

Product cited in:	Chen, Mondal, Ding, Koenig, Slaughter, Griffith, Wu: "Activation and shedding of platelet glycoprotein IIb/IIIa under non-physiological shear stress." in: **Molecular and cellular**

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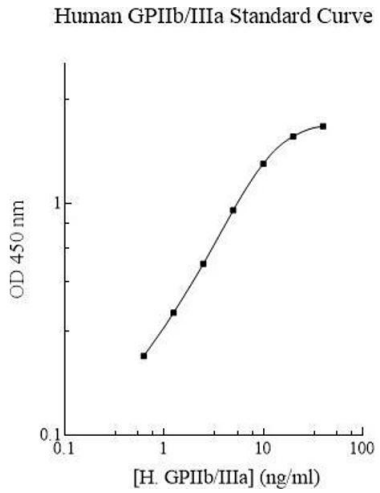
Publications

biochemistry, (2015) (PubMed).

Images



ELISA

Image 1.

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