Datasheet for ABIN579211

PDGFB ELISA Kit

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Overview

Quantity:	96 tests
Target:	PDGFB
Reactivity:	Rat
Method Type:	Sandwich ELISA
Detection Range:	31.2-2000 pg/mL
Minimum Detection Limit:	31.2 pg/mL
Application:	ELISA

Product Details

Purpose:	This immunoassay kit allows for the specific measurement of porcine platelet-derived growth factor-BB,PDGF-BB concentrations in cell culture supernates, serum, and plasma.
Sample Type:	Cell Culture Supernatant, Serum, Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes recombinant and natural Porcine PDGF-BB.
Characteristics:	Rattus norvegicus,Rat,Platelet-derived growth factor subunit B,PDGF subunit B,PDGF-2,Platelet-derived growth factor B chain,Platelet-derived growth factor beta polypeptide,Pdgfb
Components:	Reagent (Quantity): Assay plate (1), Standard 2 Sample Diluent (1 × 20ml), Assay Diluent A (1x10ml), Assay Diluent B (1x10ml), Detection Reagent A 1×120µl Detection Reagent B 1×120µl Wash Buffer (25 x concentrate) (1 × 30ml), Substrate (1 x 10ml) Stop Solution (1 x 10ml)

Target Details

Target:	PDGFB
Alternative Name:	Pdgfb (PDGFB Products)
Background:	The Platelet-Derived Growth Factor (PDGF) family consists of proteins derived from four genes (PDGF-A, -B, -C and -D) that form four disulfide-linked homodimers (PDGF-AA, -BB, -CC, -DD)and one heterodimer (PDGF-AB). These proteins and the related VEGF family proteinsshare the conserved PDGF/VEGF homology domain characterized by a pattern of highly conserved cysteine residues, which form the cysteine knot motif. PDGF-B is synthesized as a pre-pro-protein, which has a signal peptide, a mature region, an N- terminal pro-peptide, and a C-terminal extension. The C-terminal extension contains a conserved cell retention motif that confers retention of the secreted PDGF-BB within the pericellular space. Most cells produce both PDGF-A and B chains. The individual chains are assembled stochastically into disulfide-linked inactive homodimeric or heterodimeric precursors in the endoplasmic reticulum. Within the trans-Golgi network, these precursors then undergo the intracellular proteolytic processing necessary for the secretion of the biologically active mature proteins. PDGF-A and -B isoforms were originally isolated from platelets, but were subsequently found to be produced by multiple cell types including megakaryocytes, fibroblasts, keratinocytes,vascular smooth muscle cells, endothelial cells, neurons, Schwann cells, and macrophages. The mature mouse PDGF-B chain shares 98%, 89%, and 88% amino acid sequence identity to that of porcine, human, and dog, respectively. The mature mouse A and B chains share approximately 57% amino acid sequence homology. PDGF family proteins regulate diverse cellular functions by binding to and inducing the homo- or heterodimerization of two receptor subunits (PDGF Ralpha and Rbeta). Both subunits belong to the class IIIsubfamily of receptor tyrosine kinases. PDGF-BB can induce alpha / alpha, beta or/ beta homodimerization as well as/ heterodimerization.PDGF plays important roles in development and regeneration. The major source of PDGF in blood is from platelets, which releases PDGF into circulation upon platelet activation.
Gene ID:	3779
Pathways:	RTK Signaling, Fc-epsilon Receptor Signaling Pathway, EGFR Signaling Pathway, Neurotrophin Signaling Pathway, Regulation of Carbohydrate Metabolic Process, Smooth Muscle Cell Migration, Platelet-derived growth Factor Receptor Signaling

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated

Application Details

Protocol:	This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for PDGF-BB has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any PDGF-BB present is bound by the immobilized antibody. An 2 enzyme-linked polyclonal antibody specific for PDGF-BB is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of PDGF-BB bound in the initial step. The color development is stopped and the intensity of the color is measured.
Reagent Preparation:	Bring all reagents to room temperature before use. Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer. Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution of 1000 U/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high standard (1000 U/mL). The Sample Diluent serves as the zero standard (0 U/mL). Detection Reagent A and B - Dilute to the working concentration specified on the vial label using Assay Diluent A and B (1:100), respectively.
Sample Collection:	Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20 °C. Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 x g at 2 - 8 °C within 30 minutes of collection. Store samples at ≤ -20 °C. 2 Avoid repeated freeze-thaw cycles. Note: Citrate plasma has not been validated for use in this assay.
Assay Procedure:	Allow all reagents to reach room temperature. Arrange and label required number of strips. 1. Prepare all reagents, working standards and samples as directed in the previous sections. 2. Add 100 uL of Standard, Control, or sample* per well. Cover with the adhesive strip. Incubate for 2 hours at 37 °C. 3. Remove the liquid of each well, don’t wash. 4. Add 100 uL of Detection Reagent A to each well. Incubate for 1 hour at 37°C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform. 5. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (350 uL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

Application Details

	6. Add 100 uL of Detection Reagent B to each well. Cover with a new adhesive strip.Incubate for 1 hours at 37 °C. 7. Repeat the aspiration/wash as in step 5. 3 8. Add 90 uL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. Protect from light. 9. Add 50 uL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. 10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. Important Note:1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. 2. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available. 3. Duplication of all standards and specimens, although not required, is recommended. 4. When mixing or reconstituting protein solutions, always avoid foaming. 5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent. 6. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
Calculation of Results:	Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the AMA concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
Restrictions:	For Research Use only

Handling

Handling Advice:	1. The kit should not be used beyond the expiration date on the kit label.

Handling

	2. Do not mix or substitute reagents with those from other lots or sources. 3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique,incubation time or temperature, and kit age can cause variation in binding. 4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.
Storage:	4 °C/-20 °C
Storage Comment:	The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20 °C upon being received. The other reagents can be stored at 4 °C.

Publications

Product cited in:	Zhang, Wang, Yu, Liu, Qu, Wang, Gao, Zhang, Cheng: "QI-SHEN-YI-QI accelerates angiogenesis after myocardial infarction in rats." in: **International journal of cardiology**, Vol. 143, Issue 1, pp. 105-9, (2010) (PubMed).