Quantikine® ELISA

Mouse/Rat PDGF-AB Immunoassay

Catalog Number MHD00

For the quantitative determination of mouse or rat Platelet-Derived Growth Factor AB (PDGF-AB) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION	PAGE
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	2
TECHNICAL HINTS	2
MATERIALS PROVIDED & STORAGE CONDITIONS	3
OTHER SUPPLIES REQUIRED	3
PRECAUTIONS	4
SAMPLE COLLECTION & STORAGE	4
SAMPLE PREPARATION	4
REAGENT PREPARATION	5
ASSAY PROCEDURE	6
CALCULATION OF RESULTS	7
TYPICAL DATA	7
PRECISION	8
RECOVERY	8
SENSITIVITY	8
LINEARITY	9
CALIBRATION	9
SAMPLE VALUES	10
SPECIFICITY	10
REFERENCES	11
PLATE LAYOUT	12

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INTRODUCTION

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, and -DD) and one heterodimer (PDGF-AB) (1-3). These proteins and the related VEGF family proteins share the conserved PDGF/VEGF homology domain characterized by a pattern of highly conserved cysteine residues, which form the cystine knot motif. PDGF-A is synthesized as a prepro-protein with a signal peptide and an N-terminal pro-peptide (4, 5). Two PDGF-A splice variants, with or without the short stretch of conserved basic C-terminal residues, which comprises the cell retention motif, are expressed (6). The cell retention motif interacts with negatively charged glycosaminoglycans inside the cell and in the extracellular matrix. PDGF-B is also synthesized as a pre-pro-protein, which has a signal peptide, an N-terminal pro-peptide and a C-terminal extension that also contains the conserved cell retention motif (5-7). In cells that express both the -A and -B chains, the individual chains are assembled stochastically into disulfide-linked inactive homo- or hetero-dimeric precursors in the endoplasmic reticulum (1-3). Within the trans-Golgi network, these precursors then undergo intracellular proteolytic processing necessary for the secretion of the biologically active mature proteins. Secreted PDGF dimers containing the retention motif are matrix-bound and need to undergo additional proteolytic processing before they can be released from the extracellular matrix. 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 (3). The amino acid sequences of mature mouse and rat PDGF-A (short isoform) are 99% identical (4, 5). Mature mouse and rat PDGF-B also share 99% amino acid sequence identity (5, 7).

PDGF family proteins regulate diverse cellular functions by binding to and inducing the homo- or hetero-dimerization of two receptor subunits (PDGF Rα and Rβ) (1-3). Both subunits belong to the class III subfamily of receptor tyrosine kinases. PDGF-AB can induce α/α homo-dimerization as well as α/β hetero-dimerization. Ligand-induced receptor dimerization results in autophosphorylation *in trans*, resulting in the activation of several intracellular signaling pathways that have important roles in the regulation of cell growth and differentiation, as well as in wound healing.

The Quantikine Mouse/Rat PDGF-AB Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse or rat PDGF-AB in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant rat PDGF-AB and antibodies raised against recombinant rat PDGF. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse/rat PDGF-AB showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring mouse/rat PDGF-AB.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse/rat PDGF-AB has been pre-coated onto a microplate. Standards, controls, and samples are pipetted into the wells and any PDGF-AB present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse/rat PDGF-AB is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of PDGF-AB bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator 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.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- 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.
- For best results, pipette reagents and samples into the center of each well.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

<table>
<tr><th>PART</th><th>PART #</th><th>DESCRIPTION</th><th>STORAGE OF OPENED/ RECONSTITUTED MATERIAL</th></tr>
<tr><td>Mouse/Rat PDGF-AB Microplate</td><td>892828</td><td>96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse/rat PDGF-AB.</td><td>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 1 month at 2-8 °C.*</td></tr>
<tr><td>Mouse/Rat PDGF-AB Conjugate</td><td>892829</td><td>12 mL of a polyclonal antibody specific for mouse/rat PDGF-AB conjugated to horseradish peroxidase with preservatives.</td><td rowspan="10">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Mouse/Rat PDGF-AB Standard</td><td>892830</td><td>2.5 ng of recombinant rat PDGF-AB in a buffered protein base with preservatives; lyophilized.</td></tr>
<tr><td>Mouse/Rat PDGF-AB Control</td><td>892831</td><td>Recombinant rat PDGF-AB in a buffered protein base with preservatives; lyophilized. The concentration range of rat PDGF-AB after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.</td></tr>
<tr><td>Assay Diluent RD1N</td><td>895488</td><td>12 mL of a buffered protein base with preservatives.</td></tr>
<tr><td>Calibrator Diluent RD5-3</td><td>895436</td><td>2 vials (21 mL/vial) of a buffered protein base with preservatives.</td></tr>
<tr><td>Wash Buffer Concentrate</td><td>895003</td><td>21 mL of a 25-fold concentrated solution of a buffered surfactant with preservative. May turn yellow over time.</td></tr>
<tr><td>Color Reagent A</td><td>895000</td><td>12 mL of stabilized hydrogen peroxide.</td></tr>
<tr><td>Color Reagent B</td><td>895001</td><td>12 mL of stabilized chromogen (tetramethylbenzidine).</td></tr>
<tr><td>Stop Solution</td><td>895174</td><td>23 mL of diluted hydrochloric acid.</td></tr>
<tr><td>Plate Sealers</td><td>N/A</td><td>4 adhesive strips.</td></tr>
</table>

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

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- **Polypropylene** test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Platelet-poor Plasma - Collect plasma on ice using EDTA or heparin as an anticoagulant. Centrifuge at 2-8 °C for 15 minutes at 1000 x g within 30 minutes of collection. An additional centrifugation step of the separated plasma at 10,000 x g for 10 minutes at 2-8 °C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

PDGF is present in platelet granules and is released upon platelet activation. Therefore, to measure circulating levels of PDGF, platelet-free plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommmended by the Clinical and Laboratory Standards Institute (CLSI), result in incomplete removal of platelets from blood. This will cause variable and irreproducible results for assays of factors contained in platelets and released by platelet activation.

SAMPLE PREPARATION

Rat serum samples and mouse platelet-poor heparin plasma samples require a 2-fold dilution prior to assay. A suggested 2-fold dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD5-3.

Mouse serum samples require a 20-fold dilution prior to assay. A suggested 20-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent RD5-3.

Mouse EDTA platelet-poor plasma samples require a 5-fold dilution prior to assay. A suggested 5-fold dilution is 40 µL of sample + 160 µL of Calibrator Diluent RD5-3.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

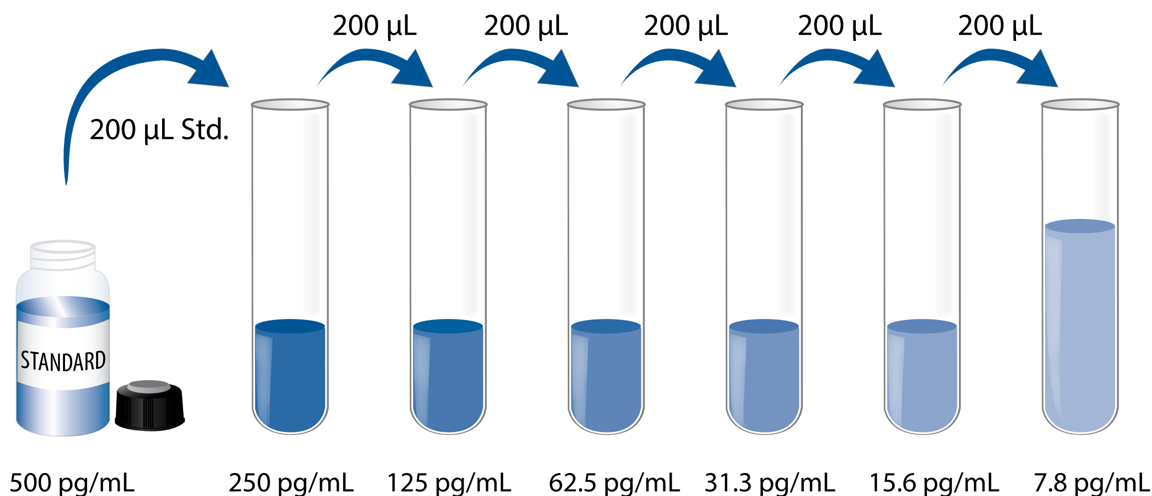
Mouse/Rat PDGF-AB Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Mouse/Rat PDGF-AB Standard - Reconstitute the Mouse/Rat PDGF-AB Standard with 5.0 mL of Calibrator Diluent RD5-3. Do not substitute other diluents. This reconstitution produces a stock solution of 500 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-3 into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse/Rat PDGF-AB Standard (500 pg/mL) serves as the high standard. Calibrator Diluent RD5-3 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, Control, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, Control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1N to each well.
4. Add 50 µL of Standard, Control, or sample* per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, 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.
6. Add 100 µL of Mouse/Rat PDGF-AB Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. 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. 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

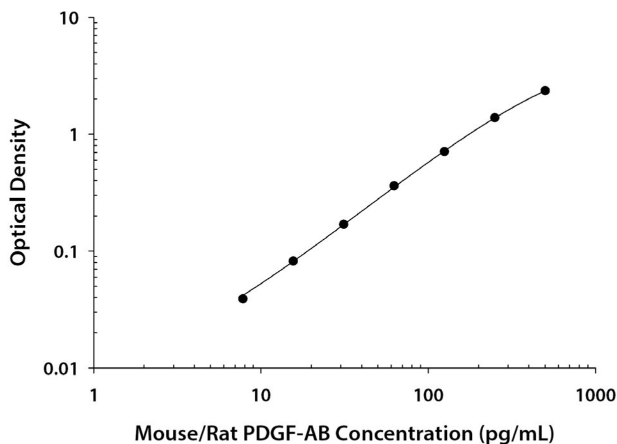
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse/rat PDGF-AB 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.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.058 0.058	0.058	—
7.8	0.095 0.098	0.097	0.039
15.6	0.136 0.143	0.140	0.082
31.3	0.227 0.227	0.227	0.169
62.5	0.413 0.424	0.419	0.361
125	0.744 0.790	0.767	0.709
250	1.425 1.457	1.441	1.383
500	2.393 2.431	2.412	2.354

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	89	127	89
Mean (pg/mL)	19	58	141	18	60	147
Standard deviation	1.4	2.9	4.6	1.8	3.6	7.4
CV (%)	7.4	5.0	3.3	10	6.0	5.0

RECOVERY

The recovery of PDGF-AB spiked to levels throughout the range of the assay in various matrices was evaluated.

Mouse Samples	Average % Recovery	Range
Cell culture supernates (n=6)	96	86-113%

Rat Samples	Average % Recovery	Range
Cell culture supernates (n=5)	95	80-108%
Serum* (n=6)	97	82-112%
EDTA plasma (n=6)	104	80-119%
Heparin plasma (n=6)	98	87-109%

*Samples were spiked and then diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Sixteen assays were evaluated and the minimum detectable dose (MDD) of PDGF-AB ranged from 0.9-3.8 pg/mL. The mean MDD was 2.3 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of PDGF-AB were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

Mouse Samples		Cell culture supernates (n=5)	Serum* (n=5)	Platelet-poor EDTA plasma* (n=5)	Platelet-poor Heparin plasma* (n=5)
1:2	Average % of Expected	101	98	97	104
	Range (%)	97-103	94-103	93-100	98-112
1:4	Average % of Expected	104	100	92	98
	Range (%)	99-108	94-107	89-95	94-105
1:8	Average % of Expected	105	97	92	95
	Range (%)	100-111	87-101	90-99	91-98
1:16	Average % of Expected	103	92	97	90
	Range (%)	99-106	80-104	89-112	88-95

Rat Samples		Cell culture supernates (n=5)	Serum* (n=5)	Platelet-poor EDTA plasma (n=5)	Platelet-poor Heparin plasma (n=5)
1:2	Average % of Expected	99	104	103	96
	Range (%)	96-102	101-107	99-106	92-98
1:4	Average % of Expected	105	107	106	95
	Range (%)	94-113	104-112	96-111	92-97
1:8	Average % of Expected	112	110	101	98
	Range (%)	106-118	102-116	92-112	93-106
1:16	Average % of Expected	107	104	107	92
	Range (%)	98-116	88-116	96-120	86-102

*Samples were diluted prior to assay as directed in the Sample Preparation section.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant rat PDGF-AB produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of PDGF-AB in this assay.

Mouse Samples	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=10)	4150	100	2706-7194
Platelet-poor EDTA plasma (n=10)	1841	100	1123-2348
Platelet-poor heparin plasma (n=10)	595	100	292-925

Rat Samples	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=10)	247	100	119-576
Platelet-poor EDTA plasma (n=10)	15	90	ND-32
Platelet-poor heparin plasma (n=10)	22	50	ND-39

ND=Non-detectable

Cell Culture Supernates - J774A.1 mouse reticulum cell sarcoma macrophage cells (1×10^6 cells/mL), were cultured for 3 days in 50 mL of DMEM supplemented with 10% fetal calf serum. An aliquot of the cell culture supernate was removed, assayed for PDGF-AB, and measured 456 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse and rat PDGF-AB.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse/rat PDGF-AB control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

FGF-8b
FGF-8c
Flt-3 Ligand
G-CSF
GM-CSF
IGF-I
IGF-II
M-CSF
PDGF Rα
PDGF Rβ
PlGF-2
$VEGF_{120}$
$VEGF_{164}$
VEGF-D
VEGF R1
VEGF R2
VEGF R3

Recombinant rat:

GM-CSF
β-NGF
PDGF-AA
PDGF-BB
VEGF

Recombinant human:

PDGF-AA
PDGF-BB
PDGF Rα
PDGF Rβ

Recombinant porcine:

GM-CSF

Natural proteins:

bovine FGF acidic
bovine FGF basic

Recombinant human PDGF-AB cross-reacts approximately 8% in this assay.

REFERENCES

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2. Li, X. and U. Eriksson (2003) Cytokine Growth Factor Rev. **14**:91.
3. Heldin, C-H. and B. Westermark (1999) Physiol. Rev. **79**:1283.
4. Rorsman, F. and C. Betsholtz (1992) Growth Factors **6**:303.
5. Herren, B. *et al.* (1993) Biochim. Biophys. Acta **1173**:294.
6. Ostman, A. *et al.* (1991) Cell Regul. **2**:503.
7. Bonthron, D.T. *et al.* (1991) Genomics **10**:287.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

For research use only. Not for use in diagnostic procedures.

NOTES

NOTES

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