Quantikine® ELISA

Mouse/Rat PDGF-BB Immunoassay

Catalog Number MBB00

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

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

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

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

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, -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 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 (4-6). 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 (1-3). 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 (3). The mature mouse PDGF-B chain shares 98%, 89%, and 88% amino acid sequence identity to that of rat (7), human (4), and dog (8), respectively. The mature mouse A and B chains share approximately 57% amino acid sequence homology (6, 9).

PDGF family proteins regulate diverse cellular functions by binding to and inducing the homo- or heterodimerization of two receptor subunits (PDGF Rα and Rβ) (1-3). Both subunits belong to the class III subfamily of receptor tyrosine kinases. PDGF-BB can induce α/α or β/β homodimerization as well as α/β heterodimerization. PDGF plays important roles in development (10) and regeneration. The major source of PDGF in blood is from platelets, which releases PDGF into circulation upon platelet activation.

The Quantikine Mouse/Rat PDGF-BB Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse/rat PDGF-BB in cell culture supernates, serum, and platelet-poor plasma. It contains *E. coli*-expressed recombinant rat PDGF-BB and antibodies raised against recombinant human PDGF-BB. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse/rat PDGF-BB 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 and rat PDGF-BB.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse/rat PDGF-BB has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any PDGF-BB present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse/rat 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. 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-BB 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 the appropriate 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.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse/Rat PDGF-BB Microplate	892860	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse/rat PDGF-BB.	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.*
Mouse/Rat PDGF-BB Conjugate	892861	12 mL of a polyclonal antibody specific for mouse/rat PDGF-BB conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse/Rat PDGF-BB Standard	892862	2 vials of recombinant rat PDGF-BB in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse/Rat PDGF-BB Control	892863	Recombinant rat PDGF-BB in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Assay Diluent RD1X	895121	11 mL of a buffered protein base with preservatives. *May contain a precipitate. Warm to room temperature. Mix well before and during use.*	
Calibrator Diluent RD5-3	895436	21 mL of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6-3	895165	21 mL of animal serum with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* 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.
- 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 a preservative 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 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 RD6-3.

Mouse serum samples require a 10-fold dilution prior to assay. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD6-3.

Mouse platelet-poor EDTA 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 RD6-3.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

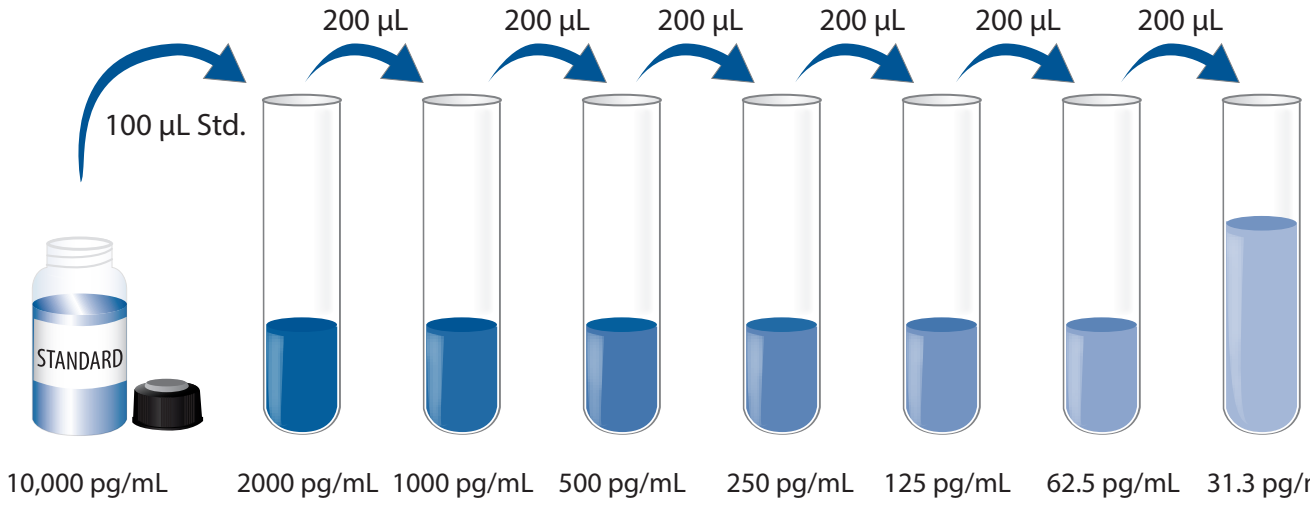
Mouse/Rat PDGF-BB Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

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-BB Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse/Rat PDGF-BB Standard with Calibrator Diluent RD5-3 (*for cell culture supernate samples*) or Calibrator Diluent RD6-3 (*for serum/plasma samples*). Do not substitute other diluents. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 400 µL of Calibrator Diluent RD5-3 (*for cell culture supernate samples*) or Calibrator Diluent RD6-3 *(for serum/plasma samples*) into the 2000 pg/mL tube. Pipette 200 µL of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube gently but thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent 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 RD1X to each well. *Assay Diluent RD1X may contain a precipitate. Mix well before and during use.*
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-BB 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.).

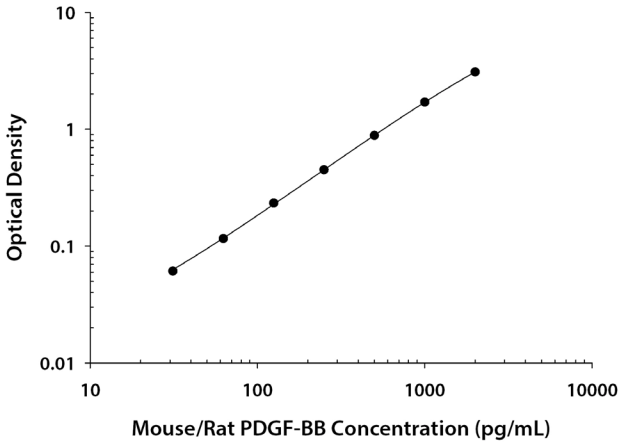
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse/rat PDGF-BB 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

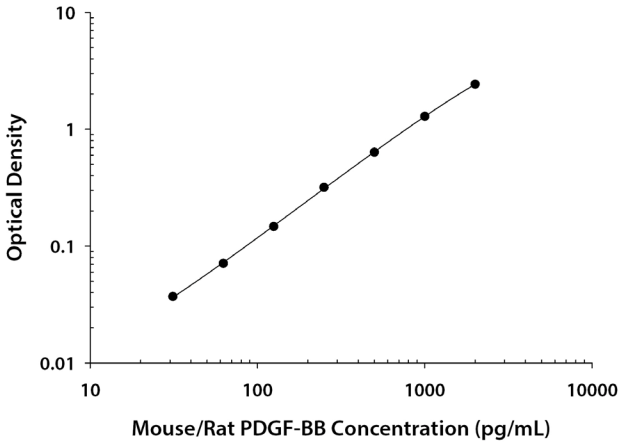
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.068 0.071	0.070	—
31.3	0.129 0.132	0.131	0.061
62.5	0.184 0.187	0.186	0.116
125	0.301 0.304	0.303	0.233
250	0.516 0.524	0.520	0.450
500	0.937 0.971	0.954	0.884
1000	1.753 1.796	1.775	1.705
2000	3.101 3.214	3.158	3.088

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.072 0.079	0.076	—
31.3	0.112 0.114	0.113	0.037
62.5	0.145 0.149	0.147	0.071
125	0.217 0.228	0.223	0.147
250	0.390 0.397	0.394	0.318
500	0.704 0.718	0.711	0.635
1000	1.359 1.369	1.364	1.288
2000	2.500 2.503	2.502	2.426

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 components.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	22	24	24
Mean (pg/mL)	49.7	252	823	52.1	246	811
Standard deviation	4.5	18.7	36.7	5.3	21.1	40.4
CV (%)	9.1	7.4	4.5	10.2	8.6	5.0

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	22	22	23
Mean (pg/mL)	61.1	298	1019	69.5	305	1048
Standard deviation	5.6	24.6	40.3	6.6	26.3	98.5
CV (%)	9.2	8.3	4.0	9.5	8.6	9.4

RECOVERY

The recovery of mouse/rat PDGF-BB spiked to levels throughout the range of the assay in various matrices was evaluated.

Mouse Samples	Average % Recovery	Range
Cell culture supernates (n=8)	100	89-118
Heparin plasma* (n=5)	96	85-113

Rat Samples	Average % Recovery	Range
Cell culture supernates (n=8)	102	94-114
Serum* (n=5)	100	89-113
EDTA plasma (n=5)	102	86-111
Heparin plasma (n=5)	102	85-111

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

LINEARITY

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

Mouse Samples		Cell culture supernates (n=5)	Serum* (n=5)	EDTA plasma* (n=5)	Heparin plasma* (n=5)
1:2	Average % of Expected	96	94	95	93
	Range (%)	86-102	93-96	93-97	89-97
1:4	Average % of Expected	102	94	92	91
	Range (%)	96-105	89-101	87-99	84-97
1:8	Average % of Expected	106	98	91	93
	Range (%)	97-111	93-104	84-102	85-106
1:16	Average % of Expected	103	90	93	94
	Range (%)	93-113	84-97	83-106	81-108

Rat Samples		Cell culture supernates (n=5)	Serum* (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	Average % of Expected	98	94	95	94
	Range (%)	90-110	89-97	91-97	87-99
1:4	Average % of Expected	98	92	91	95
	Range (%)	93-108	90-94	88-95	87-99
1:8	Average % of Expected	99	93	93	98
	Range (%)	92-112	89-104	89-97	92-109
1:16	Average % of Expected	98	92	94	99
	Range (%)	90-112	86-100	84-103	81-112

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

SENSITIVITY

Thirty-six assays were evaluated and the minimum detectable dose (MDD) of mouse/rat PDGF-BB ranged from 4.0-19.3 pg/mL. The mean MDD was 7.7 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.

CALIBRATION

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

SAMPLE VALUES

Serum/Plasma - Mouse and rat samples were evaluated for detectable levels of mouse/rat PDGF-BB in this assay.

Mouse Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum* (n=10)	12,830	10,270-17,390	2148
EDTA plasma* (n=10)	4237	2066-8210	2251
Heparin plasma* (n=10)	1045	351-1584	379

Rat Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum* (n=10)	2684	2054-3576	514
EDTA plasma (n=10)	978	506-1551	443
Heparin plasma (n=10)	1018	218-1548	486

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

Cell Culture Supernates - J744A.1 mouse reticulum cell sarcoma macrophage cells (1 x 10^6 cells/mL), were cultured for 4 days in 50 mL of DMEM supplemented with 10% fetal calf serum, stimulated with 2.5 ng/mL of LPS. An aliquot of the cell culture supernate was removed, assayed for detectable levels of mouse/rat PDGF-BB, and measured 3092 pg/mL.

SPECIFICITY

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

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-BB 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
PlGF-2
$VEGF_{120}$
$VEGF_{164}$
VEGF-D
VEGF R1
VEGF R2
VEGF R3

Recombinant rat:

GM-CSF
β-NGF
PDGF-AA
$VEGF_{164}$

Recombinant human:

PDGF-AA
PDGF-C
PDGF Rα
PDGF Rβ

Recombinant porcine:

GM-CSF

Natural proteins:

bovine FGF acidic
bovine FGF basic

Some cross-reactivity was observed with the following:

Factor	% Cross-reactivity
Recombinant rat PDGF-AB	10.2
Recombinant human PDGF-AB	5.3
Recombinant human PDGF-BB	64.7
Natural human PDGF	38.1
Natural porcine PDGF	65.2

Some interference was observed with the following:

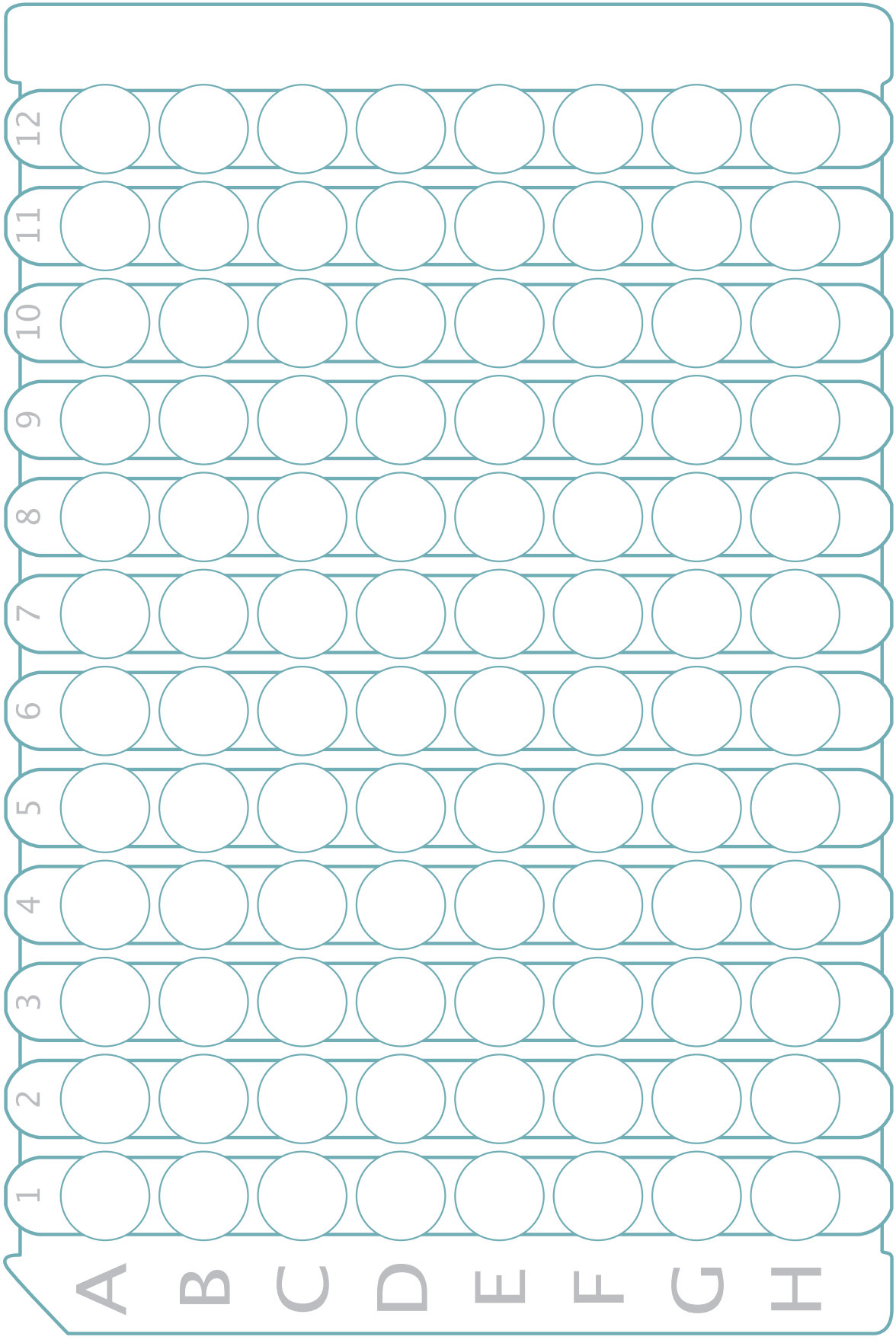
Factor	Concentration (pg/mL)
Recombinant mouse PDGF Rα	≥ 10,000
Recombinant mouse PDGF Rβ	≥ 1000
Recombinant rat PDGF Rβ	≥ 5000

REFERENCES

1. Fredriksson, L. *et al.* (2004) Cytokine and Growth Factor Rev. **15**:197.
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. Herren, B. *et al.* (1993) Biochim. Biophys. Acta **1173**:294.
5. Ostman, A. *et al.* (1991) Cell Regul. **2**:503.
6. Bonthron, D.T. *et al.* (1991) Genomics **10**:287.
7. Johnsson, A. *et al.* (1984) EMBO J. **3**:921.
8. Cartwright, J.M. (2004) GenBank Accession #:AAS60200.
9. Rorsman, F. and C. Betsholtz (1992) Growth Factors **6**:303.
10. Hoch, R.V. and P. Soriano (2003) Development **130**:4769.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES