

Quantikine[®]

Human PDGF-BB Immunoassay

Catalog Number DBB00
SBB00
PDBB00

For the quantitative determination of human platelet-derived growth factor-BB (PDGF-BB) 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.**

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INTRODUCTION

Platelet-derived growth factor (PDGF) is a glycosylated, disulfide-linked dimer (see references 1 - 3 for reviews on PDGF). There are two types of polypeptide, A (16 kDa) and B (14 kDa), with about 50% sequence identity, disulfide linked into three possible dimeric molecules, PDGF-AA, -AB and -BB. The amount of each form depends on the relative expression of the two PDGF polypeptides, which varies by type of cell. The three forms of PDGF have different but overlapping biological activities.

There are two structurally related but distinct PDGF receptors, a 170 kDa α -receptor (PDGF R α) and a 190 kDa β -receptor (PDGF R β), each with its own variation in signalling mechanism. Each subunit of PDGF binds one receptor, leading to receptor dimerization. The receptors are tyrosine kinases, and dimerization leads to autophosphorylation to activate the receptor. The signal is transmitted through a variety of intracellular signalling pathways with complex positive and negative feedbacks and with synergistic and inhibitory co-stimuli. The specificities of the two receptors differ; the α -receptor binds either the A or B polypeptide, while the β -receptor binds only the B polypeptide. With three PDGFs and two receptors, all differentially expressed, and with the complex intracellular regulatory pathways, there is a spectrum of biological actions of PDGF.

PDGF acts on many cells, especially mesenchymal cells, as a mitogen and a chemotactic factor. PDGF promotes wound healing, mediates intimal thickening associated with atherosclerosis and may contribute to neoplastic transformation and tumor pathogenesis. The major source of PDGF in human blood is platelets, where PDGF-AA and -AB are stored in α -granules and released when platelets are activated. In the absence of platelet activation, there is little PDGF in plasma, and added PDGF is cleared rapidly. PDGF-BB and -AA differ in binding to the extracellular matrix. PDGF-BB generally binds to, and is thus localized to, the cell that released it, so that its action is more restricted to the immediate site of its production (4).

The Quantikine Human PDGF-BB Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human PDGF-BB in cell culture supernates, serum, or plasma. It contains *E. coli*-expressed recombinant human PDGF-BB and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human PDGF-BB showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that the Quantikine Human PDGF-BB kit can be used to determine relative mass values for natural human PDGF-BB.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. PDGF R β /Fc chimera 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 receptor. After washing away any unbound substances, an 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.

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, dilute the samples with the appropriate Calibrator Diluent and repeat the assay. If cell culture supernate samples require large dilutions, perform an intermediate dilution with culture media and the final dilution with the appropriate Calibrator Diluent.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Description	Part #	Cat. # DBB00	Cat. # SBB00
PDGF-BB Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with recombinant human PDGF R β /Fc chimera.	890159	1 plate	6 plates
PDGF-BB Conjugate - 21 mL/vial of polyclonal antibody against PDGF-BB conjugated to horseradish peroxidase with preservatives.	890160	1 vial	6 vials
PDGF-BB Standard - 20 ng/vial of recombinant human PDGF-BB in a buffered protein base with preservatives; lyophilized.	890161	1 vial	6 vials
Assay Diluent RD1X - 11 mL/vial of a buffered protein base with preservatives. May contain crystals. Warm to room temperature and mix well to dissolve.	895121	1 vial	6 vials
Calibrator Diluent RD5K - 21 mL/vial of a buffered protein base with preservatives. <i>For cell culture supernate samples.</i>	895119	1 vial	6 vials
Calibrator Diluent RD6-3 - 21 mL/vial of animal serum with preservatives. <i>For serum/plasma samples.</i>	895165	1 vial	6 vials
Wash Buffer Concentrate - 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives.	895003	1 vial	6 vials
Color Reagent A - 12.5 mL/vial of stabilized hydrogen peroxide.	895000	1 vial	6 vials
Color Reagent B - 12.5 mL/vial of stabilized chromogen (tetramethylbenzidine).	895001	1 vial	6 vials
Stop Solution - 6 mL/vial of 2 N sulfuric acid.	895032	1 vial	6 vials
Plate Covers - Adhesive strips.	—	4 strips	24 strips

DBB00 contains sufficient materials to run an ELISA on one 96 well plate.

SBB00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDBB00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

STORAGE

Unopened Kit	Store at 2 - 8° C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Diluted Wash Buffer	May be stored for up to 1 month at 2 - 8° C.*
	Stop Solution	
	Assay Diluent RD1X	
	Calibrator Diluent RD5K	
	Calibrator Diluent RD6-3	
	Conjugate	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Standard	
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*

*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.
- Human PDGF-BB Controls (optional; available from R&D Systems).

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Caution: *Human serum used in the preparation of cell culture media may contain high levels of PDGF. Because of the low species cross-reactivity of this kit, human PDGF levels in culture media containing 10% bovine or fetal bovine serum can be assayed without interference.*

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Note: *PDGF is released during clotting; therefore, values measured in serum do not reflect levels of circulating PDGF. To determine circulating levels, platelet-poor plasma is recommended.*

Plasma - Collect plasma on ice using heparin or EDTA 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 $\leq -20^{\circ}$ 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 recommended by the National Committee for Clinical Laboratory Standards (NCCLS), 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

Serum samples require at least a 20-fold dilution into Calibrator Diluent RD6-3. A suggested 20-fold dilution is 20 μ L sample + 380 μ L Calibrator Diluent RD6-3.

REAGENT PREPARATION

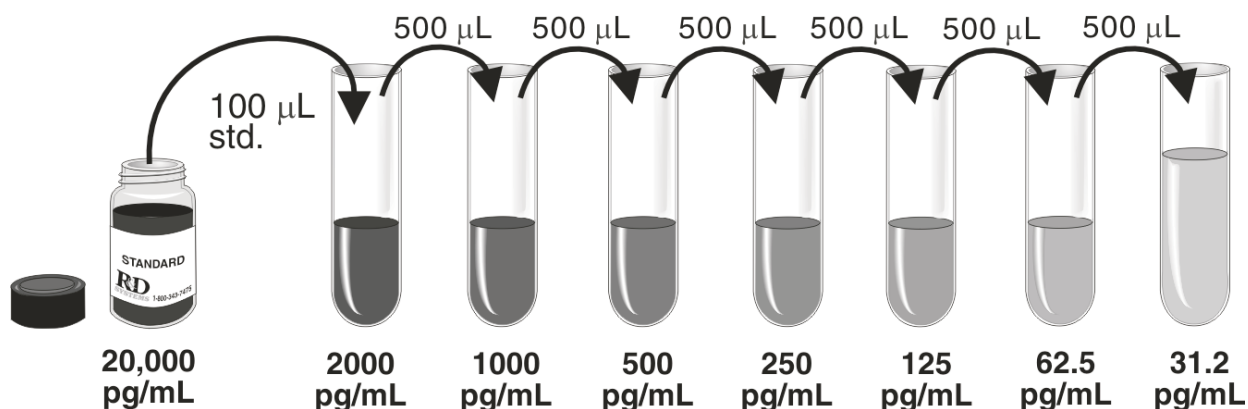
Bring all reagents to room temperature before use.

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.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μ L of the resultant mixture is required per well.

PDGF-BB Standard - Reconstitute the PDGF-BB Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 μ L of the appropriate Calibrator Diluent (*Calibrator Diluent RD5K for cell culture supernate samples or Calibrator Diluent RD6-3 for serum/plasma samples*) into the 2000 pg/mL tube. Pipette 500 μ L of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube 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 samples, standards, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, 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 100 μL of Assay Diluent RD1X to each well. Warm to room temperature and mix well to dissolve crystals before use.
4. Add 100 μL of Standard, control, or sample* per well. 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 three times for a total of four 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 toweling.
6. Add 200 μL of PDGF-BB Conjugate to each well. Cover with a new adhesive strip.
For cell culture supernate samples: Incubate for 1.5 hours at room temperature.
For serum/plasma samples: Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 μL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the well is green or if the 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. 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.

*Serum samples require dilution. See Sample Preparation section.

ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, standards, and samples as instructed.



2. Add 100 μ L Assay Diluent RD1X to each well.



3. Add 100 μ L Standard, control, or sample* to each well. Incubate 2 hours at RT.



4. Aspirate and wash 4 times.



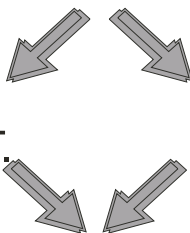
5. Add 200 μ L Conjugate to each well.

**Serum/Plasma
Samples**

**Cell Culture Supernate
Samples**

Incubate 2 hours at RT.

Incubate 1.5 hours at RT.



6. Aspirate and wash 4 times.



7. Add 200 μ L Substrate Solution to each well. Incubate 30 minutes at RT. **Protect from light.**



8. Add 50 μ L Stop Solution to each well. Read at 450 nm within 30 minutes.
 λ correction 540 or 570 nm

*Serum samples require dilution.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

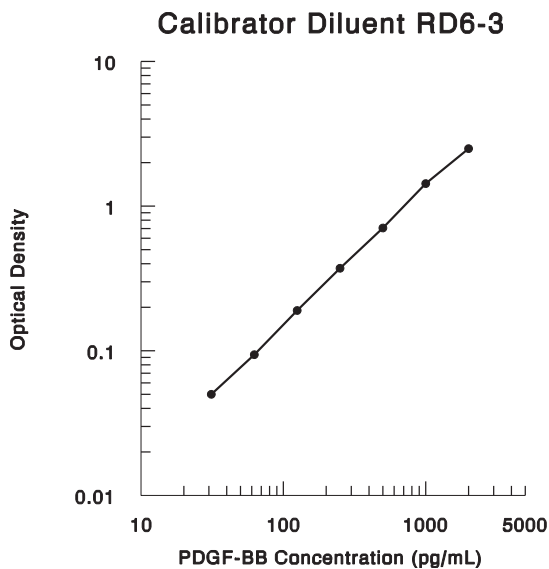
Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation.

To determine the PDGF-BB concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding PDGF-BB concentration.

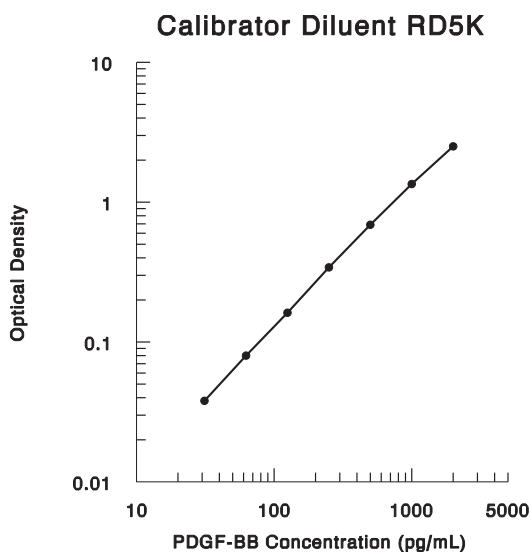
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

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



pg/mL	O.D.	Average	Corrected
0	0.033 0.032 0.078	0.032	—
31.2	0.086 0.122	0.082	0.050
62.5	0.130 0.219	0.126	0.094
125	0.226 0.406	0.222	0.190
250	0.403 0.751	0.404	0.372
500	0.726 1.494	0.738	0.706
1000	1.434 2.570	1.464	1.432
2000	2.491	2.530	2.498



pg/mL	O.D.	Average	Corrected
0	0.031 0.033 0.068	0.032	—
31.2	0.071 0.112	0.070	0.038
62.5	0.112 0.193	0.112	0.080
125	0.194 0.368	0.194	0.162
250	0.379 0.700	0.374	0.342
500	0.744 1.372	0.722	0.690
1000	1.390 2.582	1.381	1.349
2000	2.497	2.540	2.508

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.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

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 forty separate assays to assess inter-assay precision.

Serum/Plasma Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	310	759	1093	287	772	1082
Standard deviation	14.0	18.6	25.5	21.9	56.9	89.8
CV (%)	4.5	2.4	2.3	7.6	7.4	8.3

Cell Culture Supernate Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	268	639	935	251	655	918
Standard deviation	11.3	28.4	26.8	22.4	38.5	59.3
CV (%)	4.2	4.4	2.9	8.9	5.9	6.5

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n = 4)	94	86 - 102%
Serum* (n = 6)	102	93 - 114%
platelet-poor plasma		
Heparin plasma (n = 6)	103	89 - 112%
EDTA plasma (n = 6)	101	88 - 115%

*Samples were spiked and diluted 20-fold prior to assay.

LINEARITY

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

		platelet-poor plasma			
		Cell culture media (n = 4)	Serum* (n = 12)	Heparin plasma (n = 6)	EDTA plasma (n = 6)
1:2	Average % of Expected	104	100	105	105
	Range (%)	102 - 106	96 - 103	94 - 112	101 - 112
1:4	Average % of Expected	104	102	103	102
	Range (%)	101 - 106	97 - 109	98 - 107	99 - 107
1:8	Average % of Expected	102	102	101	102
	Range (%)	98 - 104	95 - 110	94 - 106	96 - 109
1:16	Average % of Expected	100	102	98	98
	Range (%)	97 - 102	91 - 112	92 - 104	92 - 105

*Samples were diluted prior to assay.

SENSITIVITY

The minimum detectable dose (MDD) of PDGF-BB is typically less than 15 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 human PDGF-BB produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of PDGF-BB in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n = 65)	3478	100	942 - 7366
platelet-poor plasma			
Heparin plasma (n = 34)	32	3	ND - 32
EDTA plasma (n = 34)	60	35	ND - 129

ND = Non-detectable

Cell Culture Supernates - Human peripheral blood mononuclear cells (5×10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal calf serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 μ g/mL PHA. Aliquots of the cell culture supernate were removed and assayed for levels of natural PDGF-BB.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND	54
Stimulated	62	152

ND = Non-detectable

SPECIFICITY

This assay recognizes recombinant and natural human PDGF-BB. The factors listed below were prepared at 50 ng/mL in the appropriate Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range recombinant human PDGF-BB control were assayed for interference. No significant cross-reactivity or interference was observed except as listed below.

Recombinant human:

ANG	IL-1ra
AR	IL-1 sRI
CNTF	IL-1 sRII
β -ECGF	IL-2
EGF	IL-2 sR α
Epo	IL-3
FGF acidic	IL-3 sR α
FGF basic	IL-4
FGF-4	IL-4 sR
FGF-5	IL-5
FGF-6	IL-5 sR α
Flt-3 Ligand	IL-5 sR β
G-CSF	IL-6
GM-CSF	IL-6 sR
sgp130	IL-7
GRO α	IL-8
GRO β	IL-9
GRO γ	IL-10
HB-EGF	IL-11
HGF	IL-12
IFN- γ	IL-13
IGF-I	KGF
IGF-II	LAP (TGF- β 1)
IL-1 α	LIF
IL-1 β	M-CSF

Recombinant mouse:

GM-CSF
IL-1 α
IL-1 β
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-13
LIF
MIP-1 α
MIP-1 β
SCF
TNF- α

Recombinant amphibian:

TGF- β 5

Natural proteins:

bovine FGF acidic
bovine FGF basic
human TGF- β 1
porcine TGF- β 1

PDGF-Related proteins:

	Cross-reactivity	
	Concentration Tested (ng/mL)	% Cross-reactivity
Recombinant Human PDGF-AB	50	0.1%
Recombinant Rat PDGF-BB	0.400	18%
Human PDGF	6.25	21%
Porcine PDGF	12.5	15%

At 200 ng/mL, α 2-macroglobulin was not found to interfere or cross-react in this assay.

REFERENCES

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2. Hughes, A.D. *et al.* (1996) *Gen. Pharmac.* **27**:1079.
3. Heldin, C.H. *et al.* (1998) *Biochem. Biophys. Acta* **1378**:F79.
4. Eming, S.A. *et al.* (1999) *Am. J. Pathol.* **154**: 281.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

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