Quantikine® ELISA

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 platelet-poor 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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Manufactured and Distributed by:

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

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

Human PDGF (Platelet-Derived Growth Factor) is a general term for a small group of structurally-related and secreted growth factors. These growth factors are widely expressed, disulfide-linked, and represent the products of four distinct genes. There are currently five named PDGFs, all of which belong to the PDGF/VEGF family, cysteine-knot superfamily of proteins. Within the PDGF family, there are two subfamilies that are characterized by either the presence, or absence, of a CUB (C1r/Cls, Urchin EGF-like, and BMP1-1) domain (1-4). Two genes (PDGF-C and -D) contain the CUB domain, while the remaining two genes (PDGF-A and -B) do not. Although all four PDGF gene products form homodimers, a PDGF-AB covalent heterodimer is also formed. Heterodimer formation appears to be a random process (5). All PDGFs are synthesized as inactive proforms that undergo intracellular or extracellular proteolytic processing to become active (3), and all PDGFs contain at least one isoform that binds heparin (2).

Human PDGF-BB is synthesized as a 35 kDa, 241 amino acid (aa) preproprecursor. It contains a 20 aa signal sequence, a 61 aa N-terminal prodomain, a 109 aa mature region (aa 82-190), and a 51 aa C-terminal prodomain (6-9). The proprecursor is initially dimerized and then intracellularly processed twice. The N-terminal domain (aa 21-81) is cleaved first, followed by cleavage of the C-terminal domain (aa 191-241). The resulting mature region is 16-17 kDa in size (or 29-32 kDa as a homodimer) (9). N-terminal glycosylation is necessary for proper folding, but is removed prior to secretion. Notably, the N-terminal prodomain remains noncovalently associated with the mature molecule after secretion, blocking any potential interactions with same-cell PDGF receptors (10). In the event the C-terminal prodomain is retained, a 40 kDa isoform may be secreted that binds to cell-surface proteoglycans (9). There is one isoform variant that contains a three aa substitution for aa 1-21. This is not secreted and assumed to be either cytoplasmic or nuclear (11). Mature human PDGF-B shares 96% and 89% aa sequence identity with canine and mouse mature PDGF-B, respectively. PDGF-BB is expressed by hepatocytes and nonresorbing osteoclasts, generating osteoblasts and bone formation (9, 12). It is also produced by platelets, macrophages, and mast cells, and at sites of injury, promotes neutrophil and macrophage infiltration for debridement, fibroblast secretion of new ECM, and IFG-I mediated re-epithelialization (13, 14).

There are a number of molecules that either directly or indirectly participate in PDGF binding. The traditional receptor(s) for PDGF is considered to be either a homodimer or heterodimer created from two 170-180 kDa type I transmembrane RTKs termed PDGF Rα and PDGF Rβ (2, 5). *In vitro* studies have established that the αα homodimer will bind PDGF-AA, -AB, -BB, and -CC, the αβ heterodimer will bind -AB, -BB and -CC, and that the ββ homodimer will bind -BB and -DD (4). *In vivo* studies have confirmed PDGF-AA and -CC acting through αα, and -BB and -DD acting through ββ (2). Other molecules that participate in PDGF binding include LRP1 which forms a complex with PDGF Rβ (15, 16), FGF R1 that complexes with both PDGF Rβ and PDGF Rα (17, 18), and neuropilin-1, which complexes with PDGF Rα (19). PDGF-BB will also bind to SorLA/LR11 and to circulating SPARC (20, 21). PDGF-A and -B gene products also bind to circulating α2-macroglobulin (22).

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 platelet-poor 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 this 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. Recombinant human PDGF Rβ/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 human 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, further dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- 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.
- 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.

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. Refer to the SDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # DBB00	CATALOG # SBB00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human PDGF-BB Microplate	890159	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with recombinant human PDGF Rβ/Fc Chimera.	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.*
Human PDGF-BB Standard	890161	2 vials	12 vials	Recombinant human PDGF-BB in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human PDGF-BB Conjugate	890160	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human PDGF-BB conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1X	895121	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives. *May contain crystals. Warm to room temperature and mix well to dissolve.*	
Calibrator Diluent RD5K	895119	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6-3	895165	1 vial	6 vials	21 mL/vial of animal serum with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

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). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human PDGF-BB Microplate	890159	50 plates
Human PDGF-BB Conjugate	890160	50 vials
Human PDGF-BB Standard	890161	25 vials
Calibrator Diluent RD5K	895119	50 vials
or		
Calibrator Diluent RD6-3	895165	50 vials
Assay Diluent RD1X	895121	50 vials
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Wash Buffer Concentrate, 25X	895126	9 bottles
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets
Package Insert	750011	2 booklets

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.
- Human PDGF-BB Controls (optional; R&D Systems®, Catalog # QC22).

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 and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Caution: *Bovine 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 up to 10% of fetal bovine serum can be assayed without interference.*

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °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.*

Platelet-poor Plasma - Collect plasma 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 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-poor plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical and Laboratory Standards Institute (CLSI), result in incomplete removal of platelets from blood.

SAMPLE PREPARATION

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

REAGENT PREPARATION

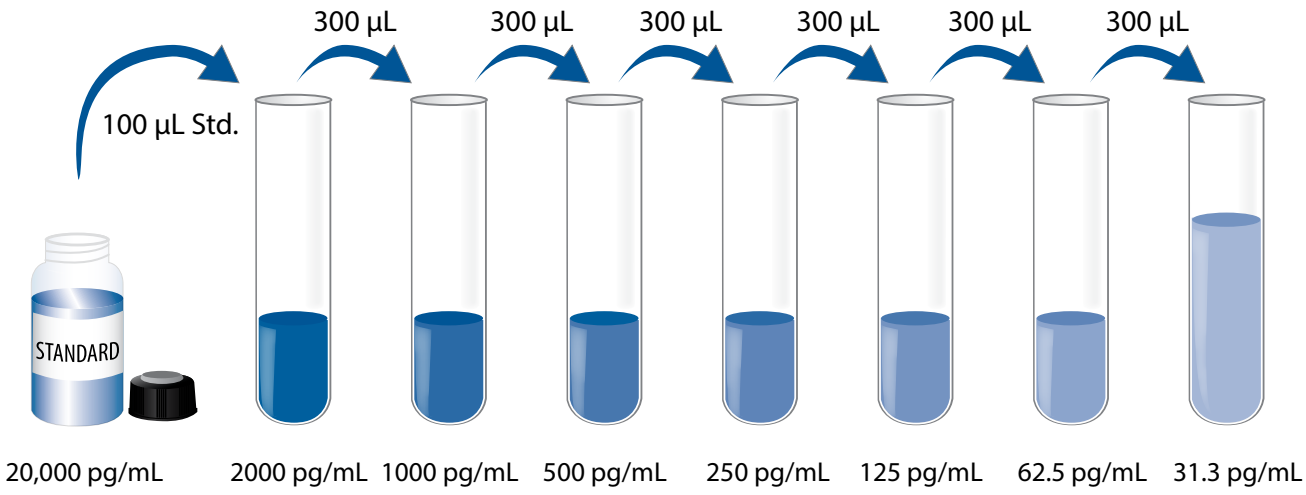
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of 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 µL of the resultant mixture is required per well.

Human PDGF-BB Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human PDGF-BB Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Mix the standard to ensure complete reconstitution by inverting vials and allow the standard to sit on benchtop for a minimum of 15 minutes.
Do not vortex standard vials and avoid constant rotation.

Pipette 900 µL of the Calibrator Diluent RD5K (*for cell culture supernate samples)* or Calibrator Diluent RD6-3 (*for serum/plasma samples*) into the 2000 pg/mL tube. Pipette 300 µL 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 standards, controls, and samples 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 Human 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.

*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.).

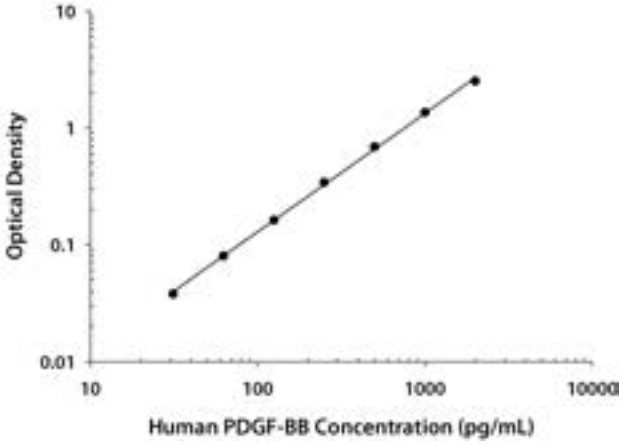
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human PDGF-BB concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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

TYPICAL DATA

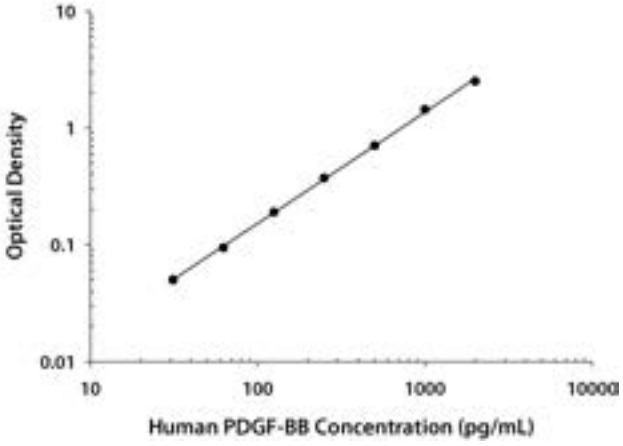
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.031 0.033	0.032	—
31.3	0.068 0.071	0.070	0.038
62.5	0.112 0.112	0.112	0.080
125	0.193 0.194	0.194	0.162
250	0.368 0.379	0.374	0.342
500	0.700 0.744	0.722	0.690
1000	1.372 1.390	1.381	1.349
2000	2.497 2.582	2.540	2.508

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.032 0.033	0.032	—
31.3	0.078 0.086	0.082	0.050
62.5	0.122 0.130	0.126	0.094
125	0.219 0.226	0.222	0.190
250	0.403 0.406	0.404	0.372
500	0.726 0.751	0.738	0.706
1000	1.434 1.494	1.464	1.432
2000	2.491 2.570	2.530	2.498

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. 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	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

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	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	2.5	2.3	7.6	7.4	8.3

RECOVERY

The recovery of human 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 EDTA plasma (n=6)	101	88-115%
Platelet-poor heparin plasma (n=6)	103	89-112%

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

SENSITIVITY

The minimum detectable dose (MDD) of human PDGF-BB is typically less than 15 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. 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 human PDGF-BB were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

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

*Samples were diluted prior to assay.

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 human 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 EDTA plasma (n=34)	60	35	ND-129
Platelet-poor heparin plasma (n=34)	32	3	ND-32

ND=Non-detectable

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

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

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant 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:

β-ECGF
EGF
FGF acidic
FGF basic
FGF-4
FGF-5
FGF-6
FGF R1α (IIIb)
FGF R1α (IIIc)
FGF R1β (IIIb)
FGF R1β (IIIc)
HB-EGF
HGF
IGF-I
IGF-II
KGF/FGF-7
LRP-1 Cluster II
LRP-1 Cluster III
LRP-1 Cluster IV
Neuropilin-1
β-NGF
PD-ECGF
PDGF-AA
PDGF-CC
PDGF Rα
SorLA
SPARC
VEGF

Other recombinants:

rat PDGF-AA
mouse PDGF-CC

At 200 ng/mL, α_2-macroglobulin was not found to interfere or cross-react in this assay.

Cross-reactivity was observed with the following:

Analyte	% Cross-reactivity
Recombinant human PDGF-AB	0.1
Recombinant rat PDGF-AB	1.5
Recombinant rat PDGF-BB	18
Human PDGF	21
Porcine PDGF	15

Interference was observed with the following:

Analyte	Interferes at levels indicated
Recombinant human PDGF Rβ	> 5 ng/mL
Recombinant human PDGF-DD	> 25 ng/mL
Recombinant mouse PDGF Rα	> 25 ng/mL
Recombinant mouse PDGF Rβ	> 200 pg/mL

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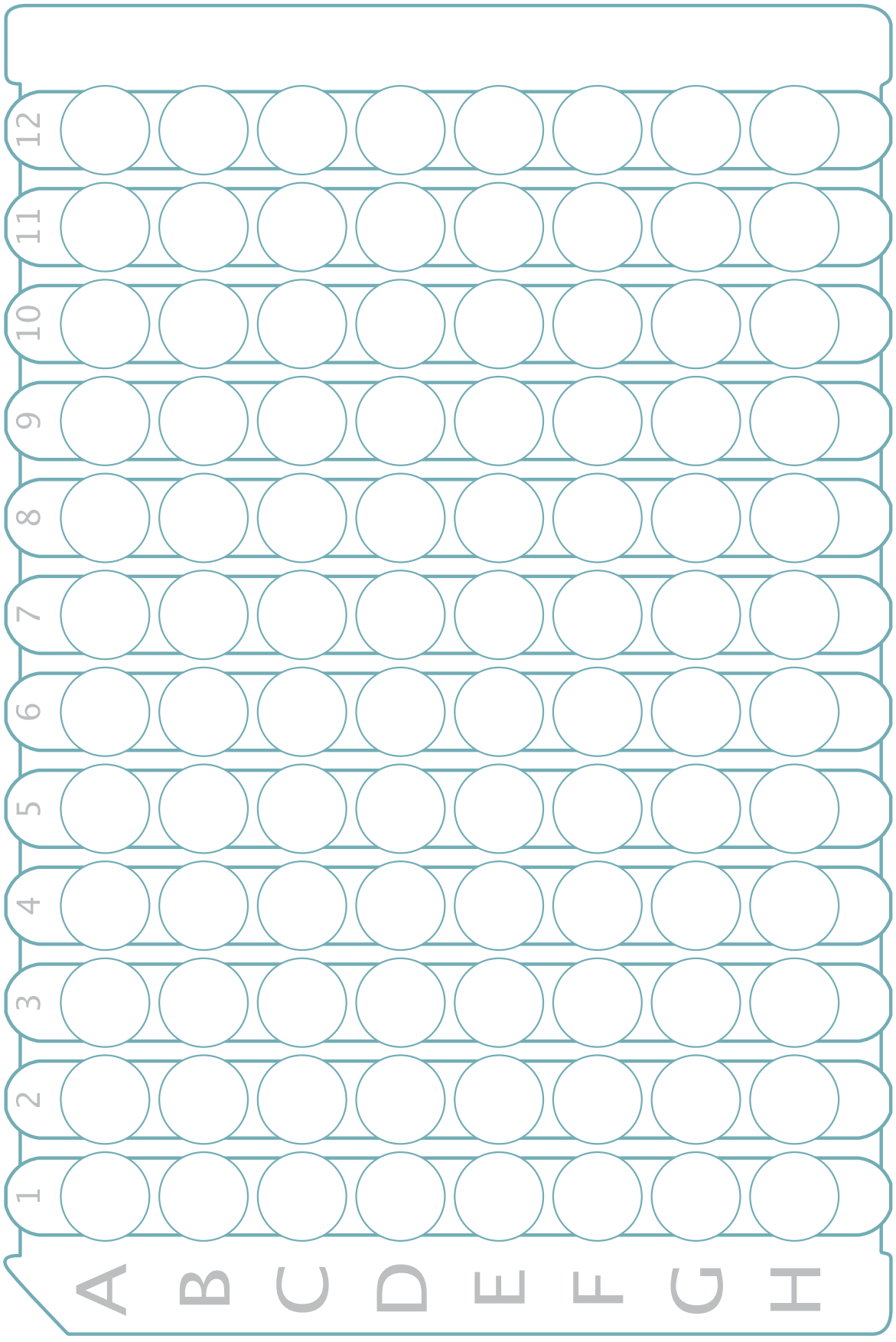
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES