Quantikine® ELISA

Human PDGF-AB Immunoassay

Catalog Number DHD00C
SHD00C
PDHD00C

For the quantitative determination of human Platelet-Derived Growth Factor AB (PDGF-AB) 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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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-AB is a 28-30 kDa, disulfide-linked heterodimer containing one 14-16 kDa PDGF-A chain and one 12-14 kDa PDGF-B chain (6, 7, 8). Although it appears to be the first PDGF isoform recognized, its presence is suggested to be unusual in that the PDGF-A and -B chains are not commonly coexpressed (2). This may be particularly true in platelets where its appearance in α-granules may be a human phenomenon (2). When, however, the -A and -B chains are coexpressed, their association, albeit random, is preferred over the formation of homodimers (5, 9). Mature PDGF-AB is formed intracellularly by the initial dimerization of PDGF-A and -B proprecursors, followed by the subsequent cleavage of each molecule's proregions (5, 10). The PDGF-A proprecursor is 24 kDa in size, and 191 amino acids (aa) in length (6, 11). Although mature 15-16 kDa PDGF-A chain is generated following cleavage after Arg86, it appears that the 8 kDa cleaved proregion remains non-covalently associated with the mature chain during secretion, acting as a block against unwanted receptor activation (12). The 31 kDa PDGF-B proprecursor is 221 aa in length (7, 10). It has two proteolytic processing sites, one after Arg81, and a second after Thr190 that may or may not be utilized. This generates a 12-14 kDa 109 aa mature molecule (aa 82-190) that also likely continues a non-covalent association with its N-terminal proregion. Mature human PDGF-A and -B share 95% and 89% aa sequence identity with mouse PDGF-A and -B, respectively. Cells known to express PDGF-AB include platelets, endothelial cells, keratinocytes, macrophages, retinal pigment epithelium and neurons (5, 13, 14). PDGF-AB has documented chemotactic properties for multiple cell types, and is also known to be mitogenic for diverse cell types. Relative to the related homodimeric isoforms, PDGF-AB will induce mitosis but will not mediate IP3 (D-myo-inositol-1,4,5-trisphosphate) production by vascular smooth muscle cells. PDGF-AA, by contrast, has no effect on either mitosis or IP3 production, while PDGF-BB induces both mitosis and IP3 production (14). Thus, it would appear that the three members (isoforms) of the non-CUB domain containing subfamily have some non-overlapping activities.

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 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 α_2-macroglobulin (22).

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

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human PDGF-BB has been pre-coated onto a microplate. Standards 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 human PDGF-AA 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-AB 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 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.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # DHD00C	CATALOG # SHD00C	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human PDGF-AB Microplate	894230	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human PDGF-BB.	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-AB Standard	894232	2 vials	12 vials	Recombinant human PDGF-AB in a buffered protein base with preservatives; lyophilized. *Refer to vial label for reconstitution volume.*	Use and discard. Use a fresh Standard for each assay.
Human PDGF-AB Conjugate	894231	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human PDGF-AA 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 a precipitate. Warm to room temperature. Mix well before and during use.*	
Calibrator Diluent RD5R	895190	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6-11	895489	2 vials	12 vials	21 mL/vial of a buffered protein base 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.

DHD00C contains sufficient materials to run an ELISA on one 96 well plate.
SHD00C (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDHD00C). 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.

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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human PDGF-AB Controls (optional; available from R&D Systems).

PRECAUTIONS

Calibrator Diluent RD6-11 contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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.

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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: *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 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.

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.*
Icteric samples are not suitable 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 Laboratory and 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

Use polypropylene tubes.

Serum samples require at least a 50-fold dilution. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD6-11.

REAGENT PREPARATION

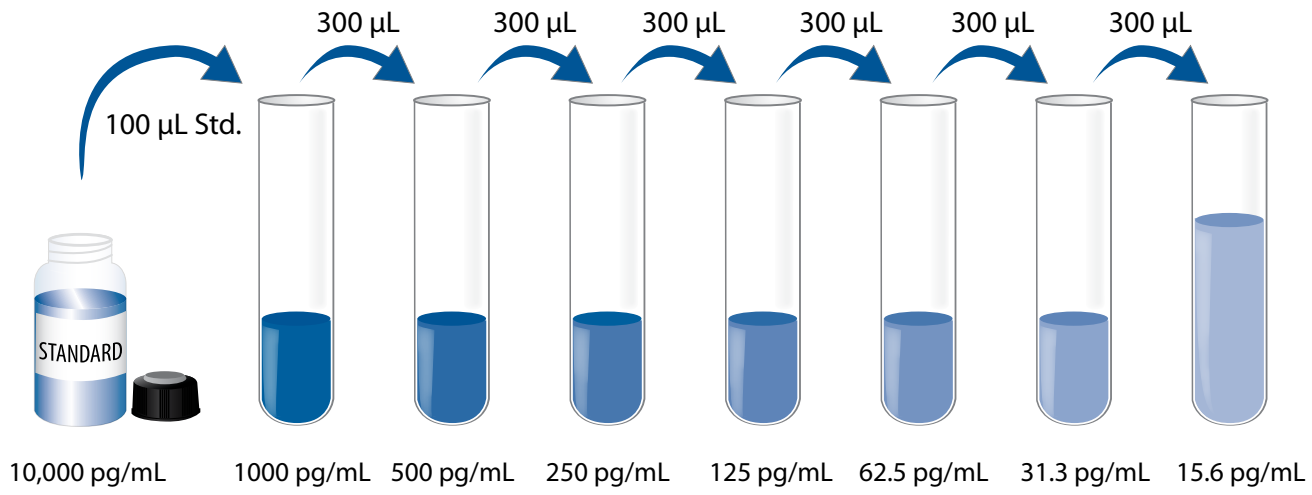
Bring all reagents to room temperature before use.

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 µL of the resultant mixture is required per well.

Human PDGF-AB Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human PDGF-AB Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5R (*for cell culture supernate samples*) or Calibrator Diluent RD6-11 (*for serum/plasma samples*) into the 1000 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 1000 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard (0 pg/mL).



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ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, 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. *RD1X may contain a precipitate. Warm to room temperature. Mix well before and during 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 on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record the 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 towels.
6. Add 200 µL of Human PDGF-AB Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. 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.

CALCULATION OF RESULTS

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

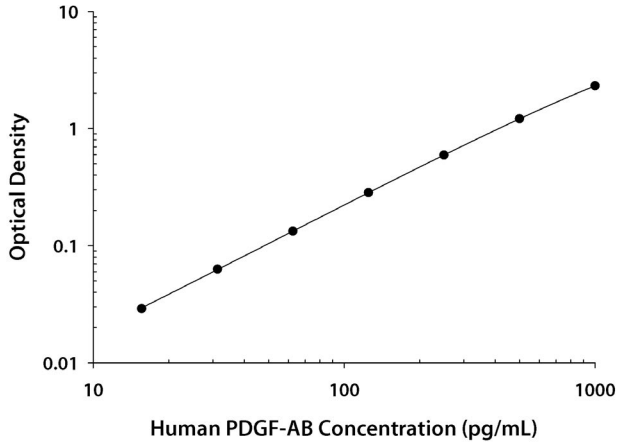
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best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

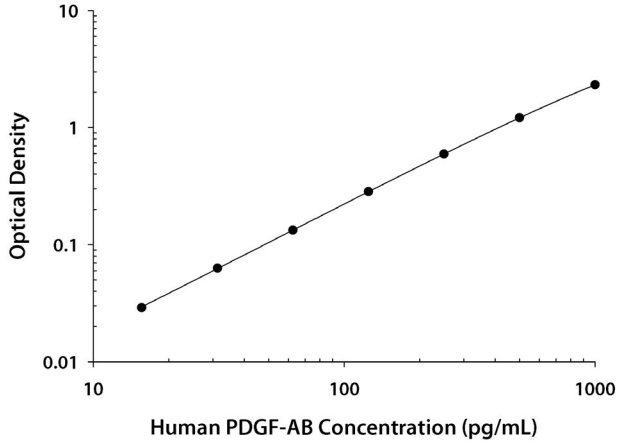
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.005 0.005	0.005	—
15.6	0.033 0.034	0.034	0.029
31.3	0.067 0.068	0.068	0.063
62.5	0.135 0.141	0.138	0.133
125	0.286 0.291	0.289	0.284
250	0.596 0.602	0.599	0.594
500	1.196 1.236	1.216	1.211
1000	2.319 2.329	2.324	2.319

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.005 0.006	0.006	—
15.6	0.035 0.036	0.036	0.030
31.3	0.070 0.070	0.070	0.064
62.5	0.142 0.146	0.144	0.138
125	0.287 0.293	0.290	0.284
250	0.598 0.605	0.602	0.596
500	1.209 1.238	1.224	1.218
1000	2.351 2.439	2.395	2.389

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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 twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of kit components.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	92.7	312	656	102	343	701
Standard deviation	8.28	9.73	15.2	10.9	29.9	52.3
CV (%)	8.9	3.1	2.3	10.7	8.7	7.5

SERUM/PLATELET-POOR PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	107	350	726	119	362	723
Standard deviation	6.95	10.9	63.1	10.7	20.9	54.5
CV (%)	6.5	3.1	8.7	9.0	5.8	7.5

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	105	93-119%
Platelet-poor EDTA plasma (n=4)	104	78-117%
Platelet-poor heparin plasma (n=4)	105	86-121%

SENSITIVITY

Seventy assays were evaluated and the minimum detectable dose (MDD) of human PDGF-AB ranged from 0.29-3.83 pg/mL. The mean MDD was 1.14 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 human PDGF-AB 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=4)	Platelet-poor EDTA plasma (n=4)	Platelet-poor heparin plasma (n=4)
1:2	Average % of Expected	92	95	97	102
	Range (%)	87-96	91-98	95-99	100-104
1:4	Average % of Expected	90	94	98	100
	Range (%)	84-93	88-99	95-105	99-101
1:8	Average % of Expected	90	92	96	103
	Range (%)	82-95	88-98	91-106	102-105
1:16	Average % of Expected	86	86	95	104
	Range (%)	75-101	74-93	90-101	101-108

*Samples were diluted prior to assay.

CALIBRATION

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

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SAMPLE VALUES

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

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum* (n=21)	14,105	6791-28,550	4888
Platelet-poor EDTA plasma (n=21)	139	43.5-610	128
Platelet-poor heparin plasma (n=21)	107	16.7-466	109

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

Cell Culture Supernates:
Human peripheral blood leukocytes (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% bovine calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the culture supernates were removed and assayed for levels of natural human PDGF-AB.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	98.2	40.8
Stimulated	43.5	79.6

4MBr-5 rhesus monkey epithelial cells were grown to confluency in F12 medium supplemented with 10% fetal calf serum, 50 ng/mL recombinant human EGF, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for levels of human PDGF-AB, and measured 211 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human 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 PDGF-AB control were assayed for interference. No significant cross-reactivity was observed, except for PDGF related proteins, as indicated. No interference was observed from any substance tested.

Recombinant human:

β-NGF
EGF
FGF acidic
FGF basic
FGF-4
FGF-5
FGF-6
FGF-9
FGF-8b
FGF-10
FGF-18
FGF R1α (IIIb)
FGF R1α (IIIc)
FGF R1β (IIIb)
FGF R1β (IIIc)
Flt-3 Ligand
G-CSF
GM-CSF
HB-EGF
HGF
IGF-I
IGF-II
KGF/FGF-7
LRP-1 Cluster II
LRP-1 Cluster III
LRP-1 Cluster IV
M-CSF
MSP/MST1
Neuropilin-1
NRG1-α/HRG1-α
NRG1-β1/HRG1-β1
PD-ECGF
PDGF-AA
PDGF-BB
PDGF-CC
PDGF-DD
PDGF Rα
PDGF Rβ
PlGF
PlGF-2
PSP94/MSMB
SorLA
SPARC
$VEGF_{121}$
$VEGF_{165}$
VEGF-D
VEGF/PlGF
VEGF R3/Flt-4

Recombinant mouse:

FGF-8c
Flt-3 Ligand
G-CSF
GM-CSF
M-CSF
PlGF-2
PDGF-CC
PDGF Rα
PDGF Rβ
$VEGF_{120}$
$VEGF_{164}$

Recombinant rat:

β-NGF
GM-CSF
PDGF-AA
PDGF-BB

Other recombinants:

porcine GM-CSF
feline VEGF

Natural proteins:

bovine FGF acidic
bovine FGF basic
porcine PDGF

Recombinant rat PDGF-AB cross-reacts approximately 29.4% in this assay.

Recombinant mouse PDGF Rα interferes at levels > 6250 pg/mL.

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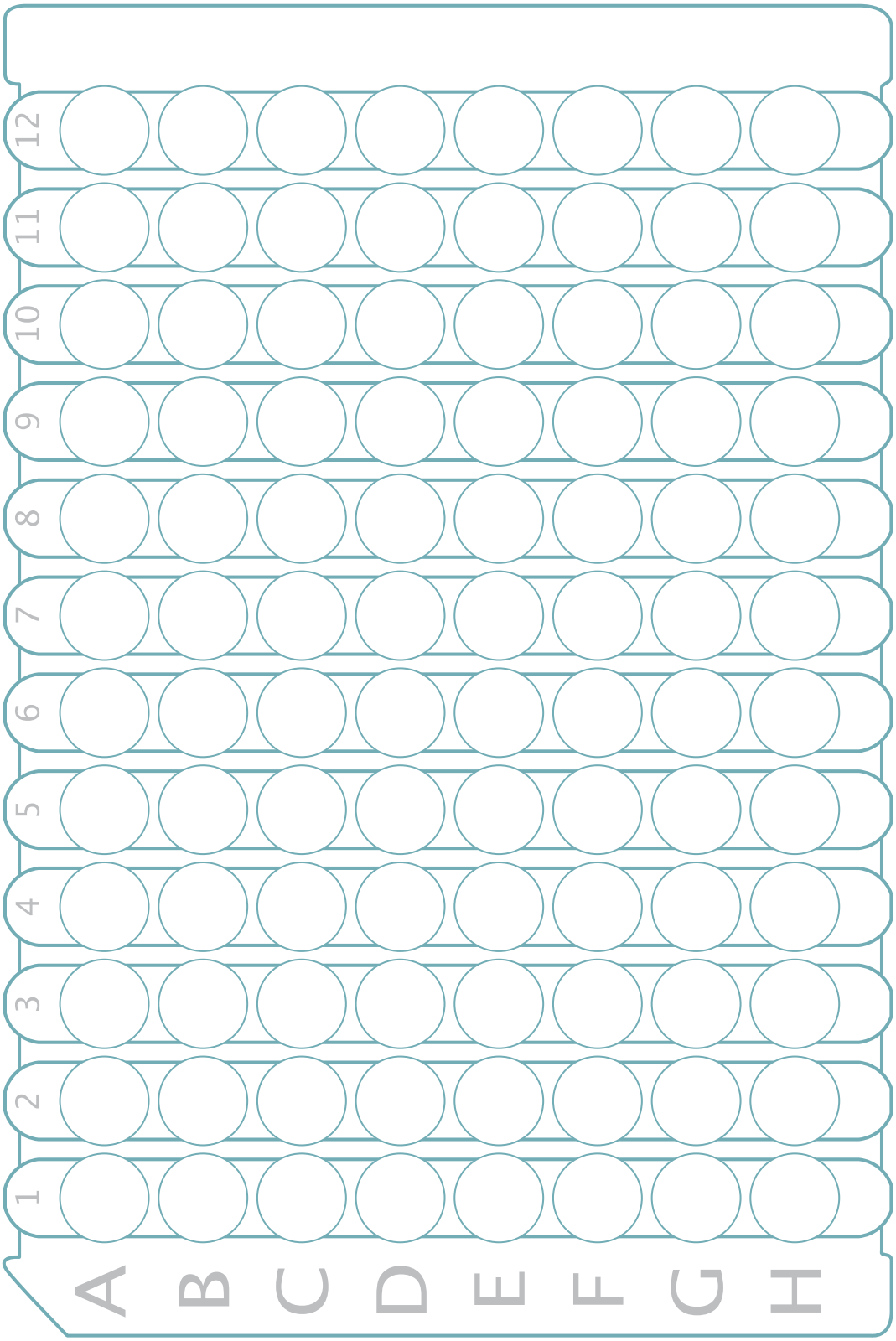
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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