# Quantikine® ELISA

## Human/Mouse PDGF-AA Immunoassay

Catalog Number DAA00B

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

**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.

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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-AA is synthesized as a 25-26 kDa, 211 amino acid (aa) preproprecursor. It contains a 20 aa signal sequence, a 66 aa N-terminal prodomain, and a 125 aa mature region (aa 87-211) (6-9). The 24-25 kDa proprecursor is initially dimerized, with each monomer proteolytically processed intracellularly into an 8 kDa N-terminal domain (aa 21-86) and a 15-16 kDa mature molecule (aa 87-211) (7, 8). Notably, the N-terminal prodomain remains noncovalently associated with the mature molecule after secretion, blocking any potential interactions with same-cell PDGF receptors (10). There is one short isoform variant that contains a three aa substitution for aa 194-211, a region that possesses a heparin-binding property. As with the 30 kDa dimeric long form, the 28 kDa dimeric short form is secreted; unlike the dimeric long form, this dimeric short form does not bind proteoglycan (11, 12). Mature human PDGF-A shares 91% and 95% aa sequence identity with mature porcine and mouse PDGF-A, respectively. PDGF-AA is expressed by monocytes, macrophages, fibroblasts and vascular smooth muscle plus endothelial cells (11, 13, 14). Functionally, PDGF-AA is suggested to act principally as a chemoattractant and inducer of protein synthesis in multiple cell types. Notably, PDGF-AA is not considered a potent mitogenic agent, an activity typically attributed to PDGF family molecules (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/Mouse PDGF-AA Immunoassay is a 4.5 hour solid-phase ELISA designed to measure PDGF-AA in human and mouse cell culture supernates, serum, platelet-poor plasma, and human saliva. It contains *E. coli*-expressed recombinant human PDGF-AA and has been shown to accurately quantitate the recombinant factor. Results obtained using natural PDGF-AA 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 human and mouse PDGF-AA.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human/mouse PDGF-AA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any PDGF-AA present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human/mouse 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-AA 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 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 calibrator diluent.
- 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 # | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|---|---|---|
| **Human/Mouse PDGF-AA Microplate** | 891049 | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human/mouse PDGF-AA. | 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.* |
| **Human/Mouse PDGF-AA Conjugate** | 893601 | 21 mL of a monoclonal antibody specific for human/mouse PDGF-AA conjugated to horseradish peroxidase with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Human/Mouse PDGF-AA Standard** | 891051 | Recombinant human PDGF-AA in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* | |
| **Assay Diluent RD1-68** | 895528 | 11 mL of a buffered protein base with preservatives. | |
| **Calibrator Diluent RD6-3** | 895165 | 21 mL of a concentrated animal serum with preservatives. *Use diluted 1:3 in this assay.* | |
| **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** | 895032 | 6 mL of 2 N sulfuric 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.
- 100 mL and 500 mL graduated cylinders.
- Collection device for saliva samples that has no enzyme binding or filtering capabilities such as a Salivette® or equivalent.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human/Mouse PDGF-AA Controls (optional; R&D Systems®, Catalog # QC22).

## PRECAUTIONS

PDGF-AA is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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 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 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 cross-reactivity, human PDGF levels in culture media containing 10% bovine or fetal bovine serum can be assayed without interference.*

**Human 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.

**Mouse Serum** - Allow blood samples to clot for 2 hours at room temperature before centrifugation 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 for 15 minutes at 2-8 °C 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-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.**

**Saliva** - Collect saliva using a collection device such as a Salivette or equivalent. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Note:** *Saliva collector must not have any enzyme binding or filtering capabilities.*

## SAMPLE PREPARATION

**Use polypropylene tubes.**

Human serum samples require a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD6-3 (diluted 1:3).

Mouse serum samples require a 20-fold dilution. A suggested 20-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent RD6-3 (diluted 1:3).

Mouse platelet-poor EDTA and heparin plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD6-3 (diluted 1:3).

Saliva samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD6-3 (diluted 1:3).

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

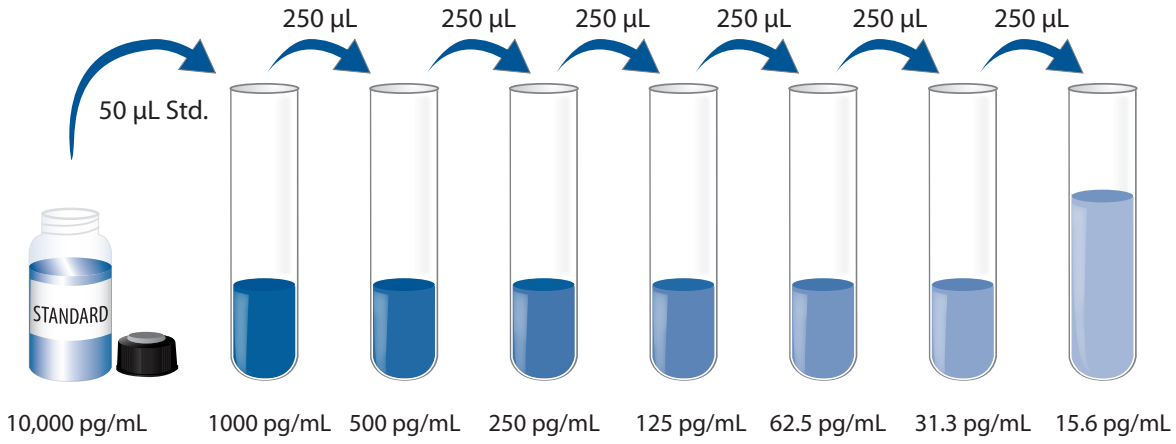
**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

**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.

**Calibrator Diluent RD6-3 (diluted 1:3)** - Add 20 mL of Calibrator Diluent RD6-3 to 40 mL of deionized or distilled water to prepare 60 mL of Calibrator Diluent RD6-3 (diluted 1:3).

**Human/Mouse PDGF-AA Standard** - **Refer to the vial label for reconstitution volume.** Reconstitute the Human/Mouse PDGF-AA 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 450 µL of Calibrator Diluent RD6-3 (diluted 1:3) into the 1000 pg/mL tube. Pipette 250 µL 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. Calibrator Diluent RD6-3 (diluted 1:3) serves as the zero standard (0 pg/mL).



## ASSAY PROCEDURE

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

**Note:** *High levels of PDGF-AA are found in saliva. Take necessary precautions (e.g. mask and gloves) to protect kit reagents.*

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 RD1-68 to each well.
4. Add 50 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 samples and standards 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 150 µL of Human/Mouse PDGF-AA 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 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 wells is green or 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

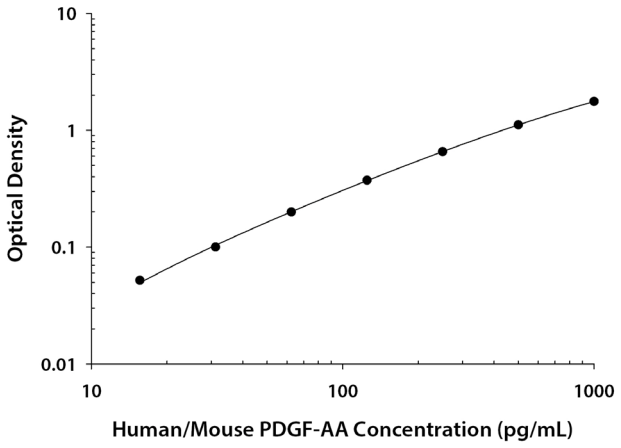
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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/mouse PDGF-AA concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

## TYPICAL DATA

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




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.037<br>0.037 | 0.037 | — |
| 15.6 | 0.084<br>0.093 | 0.089 | 0.052 |
| 31.3 | 0.130<br>0.144 | 0.137 | 0.100 |
| 62.5 | 0.235<br>0.239 | 0.237 | 0.200 |
| 125 | 0.393<br>0.427 | 0.410 | 0.373 |
| 250 | 0.688<br>0.697 | 0.693 | 0.656 |
| 500 | 1.128<br>1.166 | 1.147 | 1.110 |
| 1000 | 1.785<br>1.810 | 1.798 | 1.761 |

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

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| **Sample** | 1 | 2 | 3 | 1 | 2 | 3 |
| **n** | 20 | 20 | 20 | 24 | 24 | 24 |
| **Mean (pg/mL)** | 126 | 469 | 835 | 123 | 417 | 792 |
| **Standard deviation** | 10.9 | 38.7 | 67.4 | 14.8 | 50.6 | 83.0 |
| **CV (%)** | 8.7 | 8.3 | 8.1 | 12.0 | 12.1 | 10.5 |

## RECOVERY

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

| Sample Type | Average % Recovery | Range |
|---|---|---|
| Human cell culture media (n=4) | 97 | 80-107% |
| Human platelet-poor heparin plasma (n=4) | 93 | 83-108% |
| Human platelet-poor EDTA plasma (n=4) | 88 | 70-100% |

## SENSITIVITY

Forty-one assays were evaluated and the minimum detectable dose (MDD) of human/mouse PDGF-AA ranged from 0.85-6.29 pg/mL. The mean MDD was 2.29 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.

## CALIBRATION

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

## LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human/mouse PDGF-AA were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

| Human Samples | | Cell culture media (n=4) | Cell culture supernates (n=2) | Serum* (n=4) | Platelet-poor EDTA plasma (n=4) | Platelet-poor heparin plasma (n=4) | Saliva* (n=4) |
|---|---|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 101 | 105 | 100 | 97 | 101 | 95 |
| | Range (%) | 91-112 | 102-108 | 92-114 | 82-114 | 88-111 | 77-116 |
| 1:4 | Average % of Expected | 100 | 103 | 99 | 103 | 104 | 98 |
| | Range (%) | 88-112 | 100-104 | 92-112 | 91-122 | 99-110 | 79-109 |
| 1:8 | Average % of Expected | 98 | 103 | 99 | 101 | 104 | 110 |
| | Range (%) | 88-108 | 97-112 | 90-109 | 90-119 | 97-116 | 81-149 |
| 1:16 | Average % of Expected | 96 | 100 | 96 | 103 | 105 | 117 |
| | Range (%) | 86-104 | 95-104 | 80-115 | 98-110 | 96-122 | 94-153 |

| Mouse Samples | | Serum* (n=4) | Platelet-poor EDTA plasma* (n=5) | Platelet-poor heparin plasma* (n=2) |
|---|---|---|---|---|
| 1:2 | Average % of Expected | 101 | 105 | 102 |
| | Range (%) | 100-101 | 100-108 | 98-107 |
| 1:4 | Average % of Expected | 100 | 104 | 98 |
| | Range (%) | 95-105 | 97-112 | 95-104 |
| 1:8 | Average % of Expected | 102 | 101 | 97 |
| | Range (%) | 95-105 | 93-112 | 94-103 |
| 1:16 | Average % of Expected | 100 | 97 | 100 |
| | Range (%) | 95-108 | 82-111 | 98-102 |

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

## SAMPLE VALUES

**Serum/Plasma** - Samples were evaluated for the presence of human/mouse PDGF-AA in this assay.

| Sample Type | Mean (pg/mL) | Range (pg/mL) | Standard Deviation (pg/mL) |
|---|---|---|---|
| Human serum* (n=25) | 4208 | 2156-5818 | 1007 |
| Human saliva* (n=10) | 519 | 203-1056 | 308 |
| Mouse serum* (n=8) | 4474 | 3007-6612 | 1399 |
| Mouse platelet-poor heparin plasma* (n=8) | 180 | 131-244 | 41.6 |

| Sample Type | Mean of Detectable (pg/mL) | % Detectable | Range (pg/mL) |
|---|---|---|---|
| Human platelet-poor EDTA plasma (n=18) | 51 | 56 | ND-69.6 |
| Human platelet-poor heparin plasma (n=8) | 112 | 89 | ND-241 |
| Mouse platelet-poor EDTA plasma* (n=8) | 100 | 88 | ND-260 |

*Samples were diluted prior to assay as directed in the Sample Preparation section.
ND=Non-detectable

**Cell Culture Supernates** - Human peripheral blood leukocytes (5 x $10^6$ cells/mL) were cultured in DMEM 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 for 6 days with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of human/mouse PDGF-AA. Levels of natural PDGF-AA in stimulated and unstimulated cells were undetectable.

Other cell lines tested and the levels of human/mouse PDGF-AA observed are listed below.

| Cell Line | Value (pg/mL) |
|---|---|
| JEG-3, unstimulated | 64.7 |
| JAR, unstimulated | 61.9-840 |
| HUVEC | ND-177 |
| Jurkat | ND-105 |
| HepG2 | ND-81 |

ND=Non-detectable

## SPECIFICITY

This assay recognizes recombinant and natural human and mouse PDGF-AA.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range recombinant human PDGF-AA control were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

β-ECGF
EGF
FGF acidic
FGF basic$_{146}$
FGF-4
FGF-5
FGF-6
FGF-9
FGF-10
FGF-18
Flt-3/Flk-2 Ligand
Flt-4
G-CSF
GM-CSF
HB-EGF
HGF
HRG-α
IGF-I
IGF-II
KGF/FGF-7
M-CSF
MSP
MSPβ
β-NGF
PD-ECGF
PDGF-BB
PDGF-DD
PDGF Rα
PDGF Rβ
PlGF
VEGF$_{121}$
VEGF$_{165}$
VEGF/PlGF
VEGF-D

### Recombinant mouse:

Flt-3/Flk-2 ligand
FGF-8b
FGF-8c
G-CSF
GM-CSF
M-CSF
PDGF Rα
PDGF Rβ
PlGF-2
VEGF$_{120}$
VEGF$_{164}$

### Recombinant rat:

GM-CSF
β-NGF
PDGF-BB
VEGF$_{164}$

### Recombinant porcine:

GM-CSF

### Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF

Cross-reactivity was observed with the following:

| Recombinant Factor | Cross-reactivity |
|---|---|
| Rat PDGF-AA | 42.7% |
| Rat PDGF-AB | 1.3% |
| Mouse PDGF-CC | 0.25% |
| Human PDGF-AB | 0.13% |
| Human PDGF-CC | 0.10% |

Recombinant rat PDGF-AA shares 95.8% amino acid sequence identity with recombinant human PDGF-AA.

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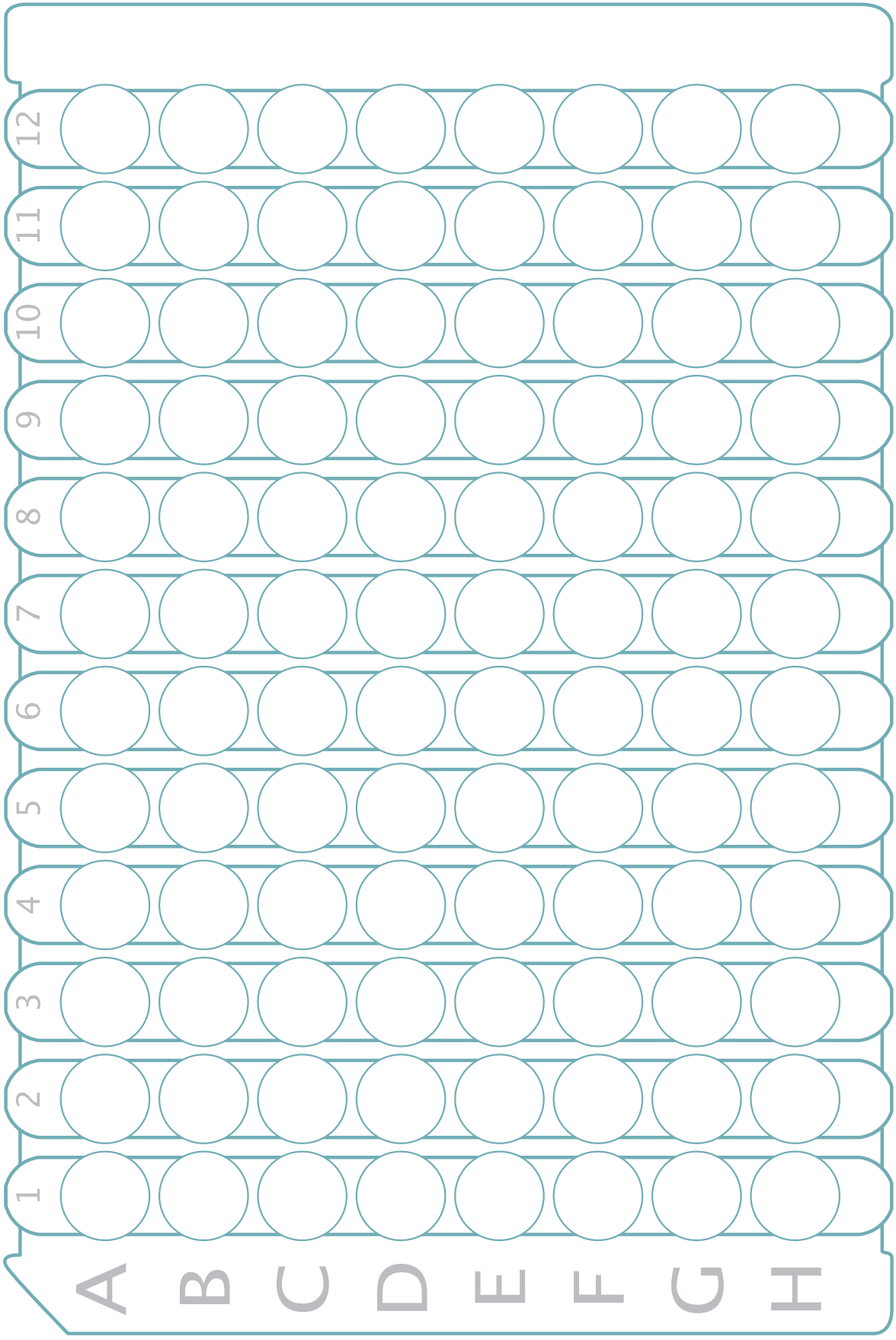
## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



## NOTES