Quantikine® ELISA

Human PDGF-CC Immunoassay

Catalog Number DCC00

For the quantitative determination of human Platelet-Derived Growth Factor CC (PDGF-CC) 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) 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-C is synthesized as a 43-48 kDa, 345 amino acid (aa) precursor. It contains a 22 aa signal sequence plus a 323 aa mature region (aa 23-345) that possesses an N-terminal CUB domain (aa 49-162) and a C-terminal PDGF region (aa 248-340) (1, 6-8). The proprecursor is secreted as a non-processed, 90 kDa latent homodimer (1, 3, 8, 9). Following release, the homodimer undergoes proteolytic cleavage, generating a bioactive 32 kDa mature molecule. Cleavage is potentially mediated by plasmin and tPA, and may occur after Arg225, 231, or 234 (1, 3, 10). In the absence of cleavage, the N-terminal CUB domain of PDGF-CC acts as a blocking structure, interfering with PDGF-CC binding to PDGF Rα and PDGF Rβ (4, 11). It also serves to anchor PDGF-CC to the ECM (2). There are two intracellular forms of PDGF-CC. One shows an alternative start site at Met164 and is termed PDGF-Cb. It is not secreted but may heterodimerize with full-length PDGF-C, generating a hybrid molecule destined for degradation (8). A second isoform is a 55 kDa (monomer) SUMOylated form of PDGF-CC that may also be found in the nucleus. SUMOylation occurs on Lys314, and an NLS is found between aa 312-322 (1, 12). Mature human PDGF-C (aa 23-345) shares 94% aa sequence identity with mature mouse PDGF-C. PDGF-CC is secreted by platelets, vascular and visceral smooth muscle cells, renal mesangial cells, macrophages and retinal pigment epithelium (1, 9, 13, 14). Functionally, PDGF-CC has documented mitogenic activity on both retinal pigment cells and fibroblasts (11, 13). It also is a chemoattractant for macrophages and endothelial cell precursors (11), and induces MMP expression by phagocytes (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), FGFR1 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-CC Immunoassay is a 4.5 hour solid phase ELISA designed to measure human PDGF-CC in cell culture supernates, serum, and platelet-poor plasma. It contains *E. coli*-expressed recombinant human PDGF-CC and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human PDGF-CC 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-CC.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for PDGF-CC has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any PDGF-CC present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for PDGF-CC 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-CC 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.
- 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
PDGF-CC Microplate	894156	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human PDGF-CC.	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.*
PDGF-CC Standard	894158	2 vials of recombinant human PDGF-CC in a buffered protein base with preservatives; lyophilized. *Refer to vial label for reconstitution volume.*	Discard after use. Use a new standard for each assay.
PDGF-CC Conjugate	894157	21 mL of a monoclonal antibody specific for human PDGF-CC conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-63	895352	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-54	895598	21 mL of a buffered protein base with preservatives. *For cell culture supernate samples. Used undiluted in this assay.*	
Calibrator Diluent RD6-15	895244	21 mL of a buffered protein base with preservatives. *For serum/plasma samples. Used diluted 1:2 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	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.
- 50 mL and 500 mL graduated cylinders.
- **Polypropylene** test tubes for dilution of standards.
- Human PDGF-CC Controls (optional; available from R&D Systems).

PRECAUTIONS

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.

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.

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.*

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. This will cause variable and irreproducible results for assays of factors contained in platelets and released by platelet activation.

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.

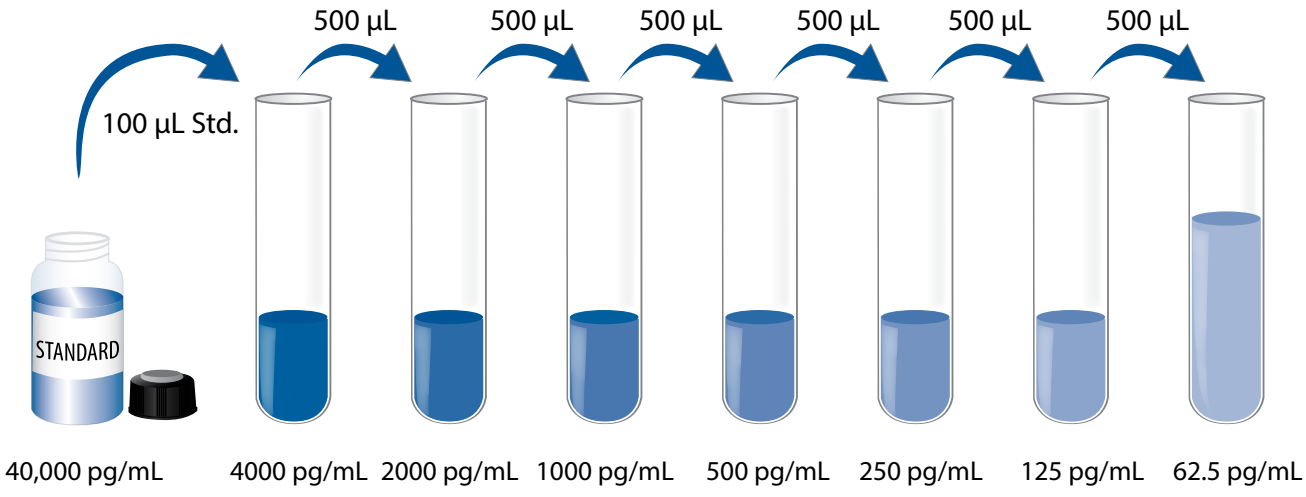
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD6-15 (diluted 1:2) - **For serum/plasma samples only.** Add 10 mL of Calibrator Diluent RD6-15 to 10 mL of deionized or distilled water to yield 20 mL of Diluted Calibrator Diluent RD6-15 (diluted 1:2).

PDGF-CC Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the PDGF-CC Standard with deionized or distilled water. This reconstitution produces a stock solution of 40,000 pg/mL. Allow the standard to sit for a minimum of 30 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5-54 (*for cell culture supernate samples*) or Calibrator Diluent RD6-15 (diluted 1:2) (*for serum/plasma samples*) into the 4000 pg/mL tube. Pipette 500 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 4000 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard (0 pg/mL).

Note: *Standard curve should be added to the microplate within 30 minutes of preparation.*



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 RD1-63 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 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 PDGF-CC 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 on the benchtop. **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.

CALCULATION OF RESULTS

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

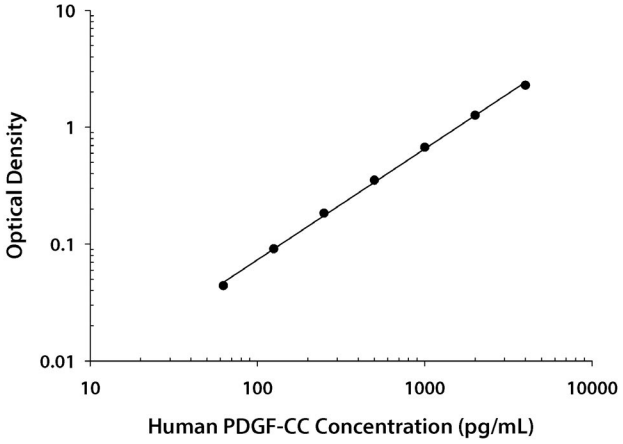
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points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

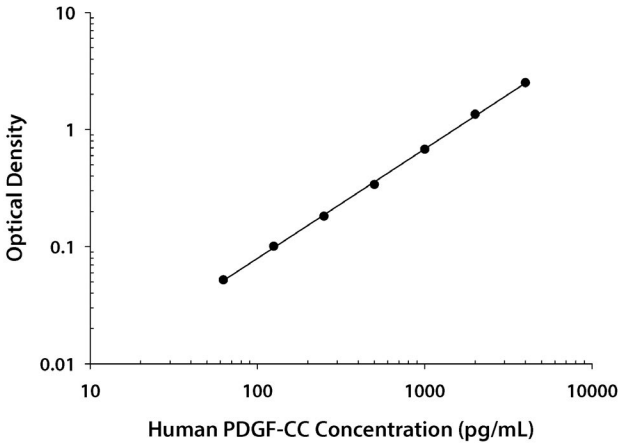
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.019 0.020	0.020	—
62.5	0.063 0.064	0.064	0.044
125	0.110 0.112	0.111	0.091
250	0.193 0.215	0.204	0.184
500	0.371 0.373	0.372	0.352
1000	0.686 0.700	0.693	0.673
2000	1.253 1.314	1.284	1.264
4000	2.300 2.308	2.304	2.284

SERUM/PLATELET-POOR PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.021 0.021	0.021	—
62.5	0.073 0.073	0.073	0.052
125	0.121 0.123	0.122	0.101
250	0.201 0.205	0.203	0.182
500	0.358 0.364	0.361	0.340
1000	0.688 0.710	0.699	0.678
2000	1.359 1.380	1.370	1.349
4000	2.495 2.562	2.529	2.508

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 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)	347	1046	2157	398	1189	2293
Standard deviation	12.2	26.4	73.6	41.5	102	160
CV (%)	3.5	2.5	3.4	10.4	8.6	7.0

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)	479	1407	2777	476	1370	2581
Standard deviation	21.7	64.1	123	48.0	104	172
CV (%)	4.5	4.6	4.4	10.1	7.6	6.7

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	92	87-97%
Serum (n=4)	99	93-108%
Platelet-poor EDTA plasma (n=4)	95	87-104%
Platelet-poor heparin plasma (n=4)	90	80-99%

SENSITIVITY

Fifty-two assays were evaluated and the minimum detectable dose (MDD) of human PDGF-CC ranged from 0.757-24.7 pg/mL. The mean MDD was 4.08 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-CC were diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum (n=4)	Platelet-poor EDTA plasma (n=4)	Platelet-poor heparin plasma (n=4)
1:2	Average % of Expected	105	100	102	103
	Range (%)	99-109	95-104	98-108	101-107
1:4	Average % of Expected	101	98	104	105
	Range (%)	92-107	94-106	102-111	99-112
1:8	Average % of Expected	102	97	106	105
	Range (%)	91-110	87-108	99-115	96-111
1:16	Average % of Expected	101	96	109	107
	Range (%)	85-112	88-102	98-114	99-114

CALIBRATION

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

SAMPLE VALUES

Serum/Platelet-poor Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human PDGF-CC 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=20)	1281	695-1963	314

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Platelet-poor EDTA plasma (n=20)	106	10	ND-149
Platelet-poor heparin plasma (n=20)	78.5	15	ND-89.3

ND=Non-detectable

Cell Culture Supernates:

OVCAR-3 human ovarian carcinoma cells were cultured in RPMI supplemented with 20% fetal bovine serum and 10 µg/mL insulin. An aliquot of the cell culture supernate was removed, assayed for levels of natural human PDGF-CC, and measured 406 pg/mL.

Capan-1 human pancreatic adenocarcinoma cells were cultured in IMDM supplemented with 20% fetal bovine serum and 4 mM L-glutamine. An aliquot of the cell culture supernate was removed, assayed for levels of natural human PDGF-CC, and measured 171 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human PDGF-CC.

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-CC 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-ECGF
EGF
FGF-4
FGF-5
FGF-6
FGF-9
FGF-10
FGF-18
FGF acidic
FGF basic (146 aa)
FGF basic (157 aa)
Flt-3/Flk-2 Ligand
G-CSF
GM-CSF
HB-EGF
HGF
IGF-I
IGF-II
KGF/FGF-7
M-CSF
MSP
MSPβ
β-NGF
NRG1-α/HRG1-α
NRG1-β1/HRG1-β1
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
PDGF Rα
PDGF Rβ
PlGF
$VEGF_{121}$
$VEGF_{165}$
VEGF/PlGF
VEGF-D
VEGF R3/Flt-4

Recombinant mouse:

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

Recombinant rat:

GM-CSF
β-NGF
PDGF-AA
PDGF-AB
PDGF-BB

Recombinant porcine:

GM-CSF

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF

Recombinant mouse PDGF-CC and recombinant human PDGF-DD cross-react approximately 0.2% in this assay.

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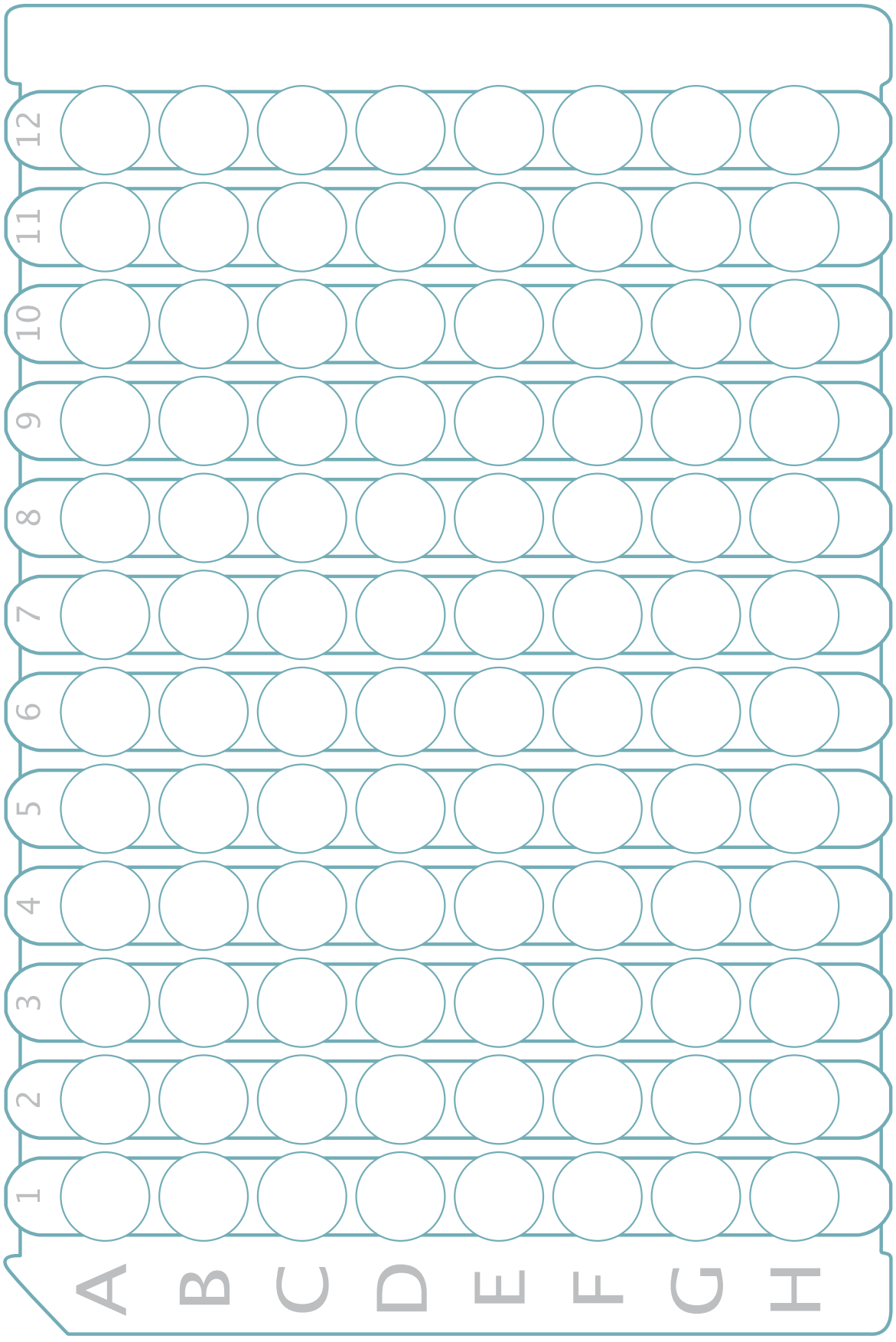
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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