Quantikine® ELISA

Human PlGF Immunoassay

Catalog Number DPG00
SPG00
PDPG00

For the quantitative determination of human placenta growth factor (PlGF) concentrations in cell culture supernates, serum, plasma, and urine.

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

Based on sequence and structural similarities, Placenta Growth Factor (PlGF) is a member of the Vascular Endothelial Growth Factor (VEGF) family that also includes VEGF/VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E (viral). PlGF is also related, albeit more distantly, to the PDGF family of growth factors (1). The VEGF family is widely known for its roles in the development and/or growth of the vascular or lymphatic endothelia (2-4). The PlGF sequence predicts a 149 amino acid (aa) mature protein with a 21 aa signal sequence and a centrally located PDGF-like domain with 8 conserved cysteine residues that form a cysteine knot structure (5). PlGF shares approximately 42% aa sequence identity with VEGF, and the two share significant structural similarity (6). Although PlGF does not share the pro-angiogenic receptor VEGF R2 with VEGF, both bind VEGF R1 (soluble and transmembrane forms), Neuropilin-1, and Neuropilin-2 (7-12). VEGF and PlGF appear to have different effects on VEGF R1 activity and, subsequently, affect the expression of different downstream genes (13). PlGF exists in at least 4 alternatively spliced forms: PlGF-1, PlGF-2, PlGF-3, and PlGF-4 (6, 14-16). Notable differences between these forms include the insertion of a heparin-binding domain in PlGF-2 and PlGF-4 that might result in increased association with the cell membrane or altered affinities for PlGF receptors (17). In mice, only the PlGF-2 ortholog has been described and shares 65% aa identity with its human counterpart (18). As the name reflects, PlGF was first identified in human placenta, and indeed, is expressed prominently in placenta under normal conditions (5, 14, 15, 19). Other tissues and cell types expressing PlGF include microvascular and human umbilical vein endothelia, bone marrow, uterine natural killer cells, and keratinocytes (17). It is also upregulated under certain pathological conditions including wound healing and tumor formation (20-23).

In comparison to VEGF, the role of PlGF in neovascularization is less clear. It can enhance the survival, growth, and migration of endothelial cells *in vitro*, and promote vessel formation in certain *in vivo* model systems (22, 24-28). However, these activities may be context-dependent (26, 29, 30). The mechanisms underlying PlGF effects on the vascular endothelia continue to be elucidated. The activity could come from the direct activation of VEGF R1 by PlGF (31). Knockout VEGF R1 does have profound effects on vascular development (32). However, only the extracellular domain appears to be necessary, suggesting the phenotype may not be due to intracellular signaling (33). PlGF can synergistically enhance VEGF-induced angiogenesis and vascular permeability (10, 34). Therefore, it has been suggested that VEGF R1 acts as a reservoir for VEGF, and that PlGF binding to the receptor displaces VEGF, freeing it to activate VEGF R2 (10). PlGF/VEGF R1 may also potentiate neovascularization via transactivation of VEGF R2 (13). In addition, PlGF is secreted as a homodimer, but may also form heterodimers with VEGF (35). Although the activity of the PlGF/VEGF heterodimer in angiogenesis is unclear, it may induce an active VEGF receptor heterodimer consisting of VEGF R1 and VEGF R2 (13, 29, 36). Monocytes/macrophages, and the molecules they secrete, have also been implicated in neovascularization. PlGF stimulates monocyte activation and chemotaxis *in vitro*, and a monocyte-mediated mechanism has been implicated in PlGF-induced arteriogenesis *in vivo* (26, 37).

Elevated levels of PlGF expression in placenta, and more specifically in the villous cytotrophoblasts and the syncytiotrophoblast, may indicate a role for PlGF in placenta formation (17, 38, 39). *In vitro*, PlGF stimulates trophoblast signal transduction including activating PLC-γ and SAPK (31, 40). In addition, PlGF can protect trophoblasts from growth factor withdrawal-induced apoptosis (31). However, embryogenesis appears to be normal in PlGF knockout mice, suggesting that the role in placentation is limited, that a redundancy exists, or that there are fundamental differences between the activities of human and mouse PlGF (17, 34). Levels of PlGF fluctuate during human pregnancy. Circulating levels of PlGF increase during the first 29-32 weeks of pregnancy and decrease thereafter (41). PlGF has also been used as a predictor of the common pregnancy-associated hypertensive disorder preeclampsia. Plasma, serum, and urine PlGF levels decrease significantly in women with preeclampsia, and/or those who subsequently develop the disorder (17, 41-44).

The Quantikine Human PlGF Immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure human PlGF in cell culture supernates, serum, plasma, and urine. The immunoassay kit contains *E. coli*-expressed recombinant human PlGF and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate recombinant human PlGF accurately. Results obtained using natural human PlGF showed dose response curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards. These results indicate that the Quantikine Human PlGF Immunoassay kit can be used to determine relative mass values for natural human PlGF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human PlGF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any PlGF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human PlGF 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 PlGF 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 from other lots or sources.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- 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.
- 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 # DPG00	CATALOG # SPG00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human PlGF Microplate	890509	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human PlGF.	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 PlGF Standard	890511	2 vials	12 vials	Recombinant human PlGF in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard the PlGF stock solution after 4 hours. Use a fresh standard for each assay.
Human PlGF Conjugate	890510	1 vial	6 vials	21 mL/vial of polyclonal antibody specific for human PlGF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-22	895490	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives. *May contain crystals. Mix well to resuspend before using.*	
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 RD6-11	895489	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For serum/plasma/urine 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.

DPG00 contains sufficient materials to run an ELISA on one 96 well plate.
SPG00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDPG00). 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.
- Test tubes for dilution of standards.
- Human PlGF Controls (optional; R&D Systems, Catalog # QC22).

PRECAUTIONS

Some components of this kit contain 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.

SAMPLE COLLECTION & STORAGE

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.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Grossly hemolyzed or lipemic samples are not suitable for measurement of human PlGF with this assay.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

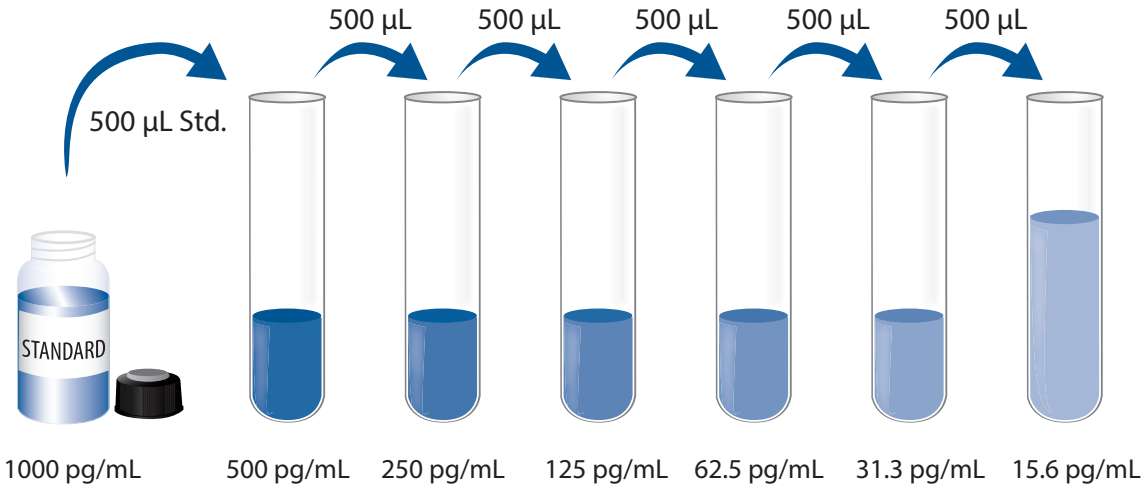
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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 RD6-11 (diluted 1:2) - **For urine samples only.** Add 10 mL of Calibrator Diluent RD6-11 to 10 mL of deioinized or distilled water to prepare 20 mL of Calibrator Diluent RD6-11 (diluted 1:2).

Human PlGF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human PlGF Standard with Calibrator Diluent RD5K (*for cell culture supernate samples*), Calibrator Diluent RD6-11 (*for serum/plasma samples*), or Calibrator Diluent RD6-11(diluted 1:2) (*for urine samples*). This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of the appropriate Calibrator Diluent into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (1000 pg/mL). 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, samples, and controls be assayed in duplicate.

1. Prepare all reagents, samples, and working standards 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 RD1-22 to each well. Assay Diluent RD1-22 may contain crystals. Warm to room temperature and mix well to resuspend before using.
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 as a record of 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 PlGF Conjugate to each well. Cover with a new adhesive strip.
   For Cell Culture Supernate Samples: Incubate for 1 hour at room temperature.
   For Serum/Plasma/Urine 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 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.).

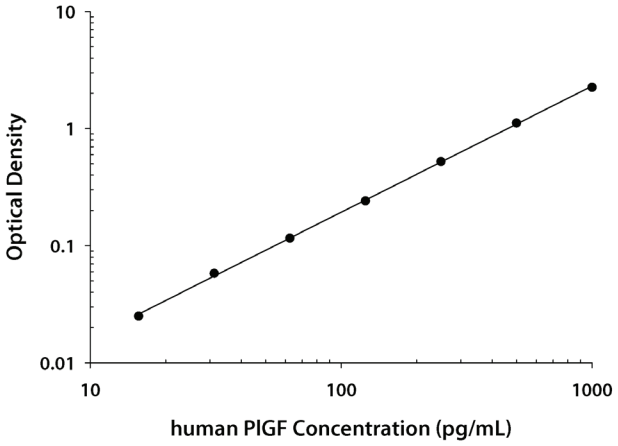
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points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

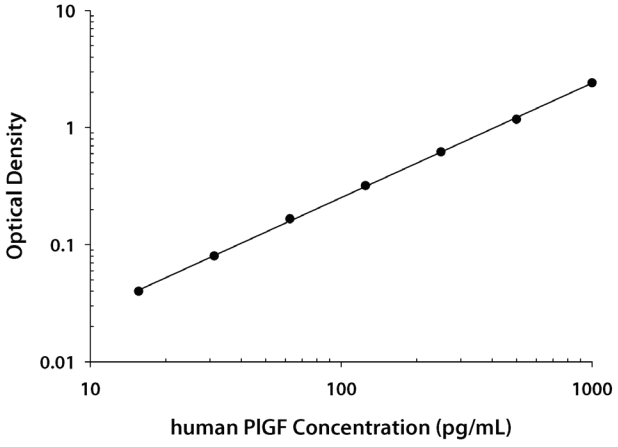
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.051 0.052	0.052	——
15.6	0.077 0.077	0.077	0.025
31.3	0.111 0.108	0.110	0.058
62.5	0.165 0.172	0.168	0.116
125	0.285 0.301	0.293	0.241
250	0.566 0.584	0.575	0.523
500	1.149 1.186	1.168	1.116
1000	2.304 2.296	2.300	2.248

SERUM/PLASMA/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.044 0.040	0.042	——
15.6	0.086 0.077	0.082	0.040
31.3	0.130 0.113	0.122	0.080
62.5	0.219 0.196	0.208	0.166
125	0.365 0.357	0.361	0.319
250	0.669 0.654	0.662	0.620
500	1.210 1.222	1.216	1.174
1000	2.589 2.323	2.456	2.414

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)	40.9	138	541	46.9	149	555
Standard deviation	2.4	7.3	38.2	6.4	13.5	32.9
CV (%)	5.9	5.3	7.1	13.6	9.1	5.9

SERUM/PLASMA/URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	54.3	175	658	55.0	184	724
Standard deviation	3.8	6.3	36.9	6.5	20.3	78.9
CV (%)	7.0	3.6	5.6	11.8	11.0	10.9

RECOVERY

The recovery of human PlGF 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=5)	97	82-107%
Serum (n=5)	96	88-118%
EDTA plasma (n=5)	102	92-117%
Heparin plasma (n=5)	94	81-113%
Citrate plasma (n=5)	99	93-120%
Urine (n=4)	97	85-112%

SENSITIVITY

The minimum detectable dose (MDD) of human PlGF is typically less than 7 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 were spiked with high concentrations of human PlGF in various matrices and diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=5)	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)	Citrate plasma (n=5)	Urine (n=5)
1:2	Average % of Expected	101	100	102	101	101	109
	Range (%)	97-104	94-106	98-106	97-108	96-104	103-112
1:4	Average % of Expected	103	103	98	101	99	107
	Range (%)	96-109	96-113	93-100	94-110	81-107	101-112
1:8	Average % of Expected	102	101	98	99	100	106
	Range (%)	93-107	87-110	88-108	91-112	84-107	94-113
1:16	Average % of Expected	103	95	94	91	103	108
	Range (%)	93-114	87-110	89-102	86-96	83-118	107-110

CALIBRATION

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

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human PlGF in this assay. No medical histories were available for the donors used in this study. See references 41, 42, and 45 for information regarding PlGF levels during pregnancy.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=95)	18	35	ND-26
EDTA Plasma (n=35)	19	26	ND-24
Citrate Plasma (n=35)	16	6	ND-17
Heparin Plasma (n=35)	22	6	ND-26
Urine (n=18)	34	39	ND-54

ND=Non-detectable

Cell Culture Supernates:

Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and stimulated for 1 and 5 days with 10 µg/mL PHA. Aliquots of the culture supernate were removed on days 1 and 5 and assayed for levels of human PlGF. All samples measured less than the lowest Human PlGF Standard, 15.6 pg/mL.

JAR human placental choriocarcinoma cells were grown in DMEM with 10% fetal bovine serum for 4 days. The measured value was 4320 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human PlGF.

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 rhPlGF control and were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ANG
AR
CNTF
EGF
Epo
FGF acidic
FGF basic
FGF-4
FGF-5
FGF-6
FGF-7
G-CSF
GM-CSF
HB-EGF
HGF
IFN-γ
IGF-I
IGF-II
IL-1α
IL-1β
IL-1 ra
IL-1 RI
IL-1 RII
IL-2
IL-2 Rα
IL-3
IL-3 Rα
IL-4
IL-4 R
IL-5
IL-5 Rβ
IL-6
IL-6 R
IL-7
IL-8
IL-9
IL-10
IL-11
IL-12
IL-13
LAP (TGF-β1)
LIF
M-CSF
β-NGF
OSM
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
SCF
TGF-α
TGF-β1
TGF-β3
TGF-β RII
TNF-α
TNF-β
sTNF RI
sTNF RII
VEGF

Recombinant mouse:

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

Recombinant human PlGF/VEGF heterodimer cross-reacts 5% in this assay.

Recombinant human PlGF-2 cross-reacts 50% in this assay.

Recombinant human VEGF R1/Flt-1/Fc Chimera was found to interfere at concentrations greater than 2000 pg/mL.

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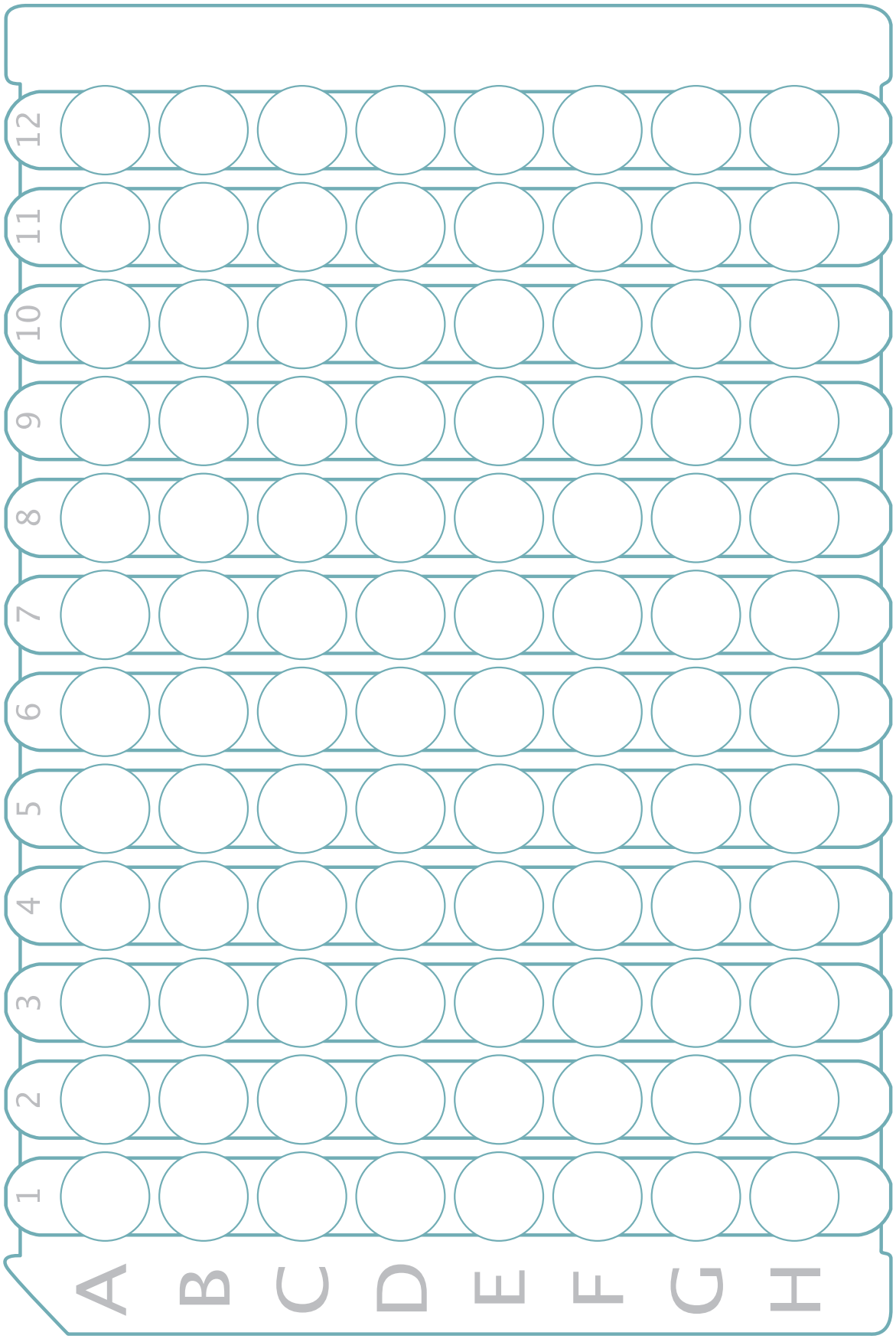
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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