Quantikine® ELISA

Mouse PlGF-2 Immunoassay

Catalog Number MP200

For the quantitative determination of mouse Placenta Growth Factor 2 (PlGF-2) concentrations in cell culture supernates, serum, and 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

Placenta growth factor (PlGF, also called PGF) is a member of the PDGF/VEGF family of secreted growth factors that share a conserved pattern of eight cysteines (1-4). Alternative splicing may result in human mature PlGF forms containing 131 (PlGF-1), 152 (PlGF-2), 203 (PlGF-3) and 224 (PlGF-4) amino acids (aa) (1-6). PlGF-2 and PlGF-4 contain a highly basic heparin-binding 21 amino acid insert at the C-terminus that is not present in PlGF-1 or -3 (2-5). In the mouse, only one PlGF that is the equivalent of human PlGF-2 has been identified (1, 5, 6). Mouse PlGF-1 shares 60%, 92%, 62% and 59% aa identity with the appropriate isoform of human, rat, canine, and equine PlGF. PlGF is mainly found as variably glycosylated 55-60 kDa disulfide-linked homodimers (2, 4). Mammalian cells expressing PlGF include villous trophoblasts, decidual cells, erythroblasts, keratinocytes, and some endothelial, smooth muscle, alveolar epithelial and cancer cells (3-5, 7-9). PlGF expression is generally low, but is upregulated in situations that require angiogenesis and inflammatory cell recruitment such as pregnancy, ischemia, wound healing, bone fracture repair, and cancer metastasis (5, 10-12).

PlGF binds to and signals through VEGF R1/Flt-1, but not VEGF R2/Flk-1/KDR (5, 6, 13, 14). In contrast, VEGF binds both but sends angiogenic signals only through VEGF R2 (13-15). PlGF and VEGF therefore compete for binding to VEGF R1, and increased PlGF expression discourages VEGF/VEGF R1 binding and promotes VEGF/VEGF R2-mediated angiogenesis (3, 4, 13, 14). PlGF (especially PlGF-1) and some forms of VEGF can form dimers, which are reported to have angiogenic activity that is anywhere from very little to being almost as potent as VEGF homodimers (4, 12-17). PlGF-2, but not PlGF-1, shows heparin-dependent binding of neuropilin (Npn)-1 and Npn-2 (4, 18, 19).

PlGF mediates compensatory responses to injury, but can also contribute to pathologies (12). PlGF induces migration of cells such as monocytes, macrophages, endothelial cells, osteogenic precursors, and dermal fibroblasts, and induces monocyte production of inflammatory cytokines and VEGF (2-5, 7, 20-23). These activities facilitate wound and bone fracture healing (4, 10, 11, 22). PlGF induction is reduced in diabetic wounds, causing impaired healing (23). PlGF may also promote chronic inflammation, which contributes to plaque formation in atherosclerosis (5, 7, 8). In active sickle cell disease, PlGF produced by erythroblasts in response to hypoxia creates a chronic inflammatory state. Increased circulating PlGF also correlates with pulmonary hypertension and vascular occlusion in sickle cell disease (7, 24-26). Increased PlGF expression by some tumors, such as breast and gastric carcinomas, generally correlates with tumor stage and metastasis, and supports tumor angiogenesis and differentiation of recruited macrophages to the tumor-associated M2 phenotype (4, 27-30). PlGF can also be immunosuppressive by inhibiting dendritic cell function, which subsequently downregulates Th1 immune response (31).

PlGF cooperates with VEGF to induce endothelial cell growth and inhibit apoptosis, thus supporting angiogenesis (11, 17, 32, 33). Circulating PlGF increases during pregnancy, reaching a peak in mid-gestation (35). Attenuation of this increase is found in preeclampsia and is thought to contribute to its pathology (9, 12, 34-37). However, deletion of PlGF in the mouse does not affect development or reproduction (4, 13). Mice lacking PlGF show normal embryonic development, but postnatal angiogenic response to ischemia is impaired (4, 13). PlGF mediates beneficial angiogenesis and cardiac hypertrophy in response to myocardial infarction; however, highest levels of plasma PlGF may correlate with the severity of heart failure (33, 38-40).

The Quantikine Mouse PlGF-2 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse PlGF-2 levels in cell culture supernates, serum, and plasma. It contains *Sf* 21-expressed recombinant mouse PlGF-2 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse PlGF-2 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 naturally occurring mouse PlGF-2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse PlGF-2 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any mouse PlGF-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse PlGF-2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of mouse PlGF-2 bound in the initial step. The sample values are then read off the standard curve.

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.
- 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.
- 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.

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
Mouse PlGF-2 Microplate	890871	96 well microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse PlGF-2.	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.*
Mouse PlGF-2 Standard	890872	2 vials of recombinant mouse PlGF-2 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard within 8 hours of reconstitution. Use a new standard and control for each assay.
Mouse PlGF-2 Control	890189	2 vials of mouse PlGF-2 in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Mouse PlGF-2 Conjugate	890873	12 mL of a polyclonal antibody against mouse PlGF-2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-47	895524	12 mL of a buffered protein solution with preservatives.	
Calibrator Diluent RD5-17	895512	21 mL of a buffered protein solution with preservatives.	
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	895174	23 mL of diluted hydrochloric 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.
- 500 mL graduated cylinder.
- **Polypropylene** test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. 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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging 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.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. 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.*
Grossly hemolyzed or lipemic samples are not suitable for use in this assay.

SAMPLE PREPARATION

Use polypropylene tubes.

Serum and plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD5-17. Mix well.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

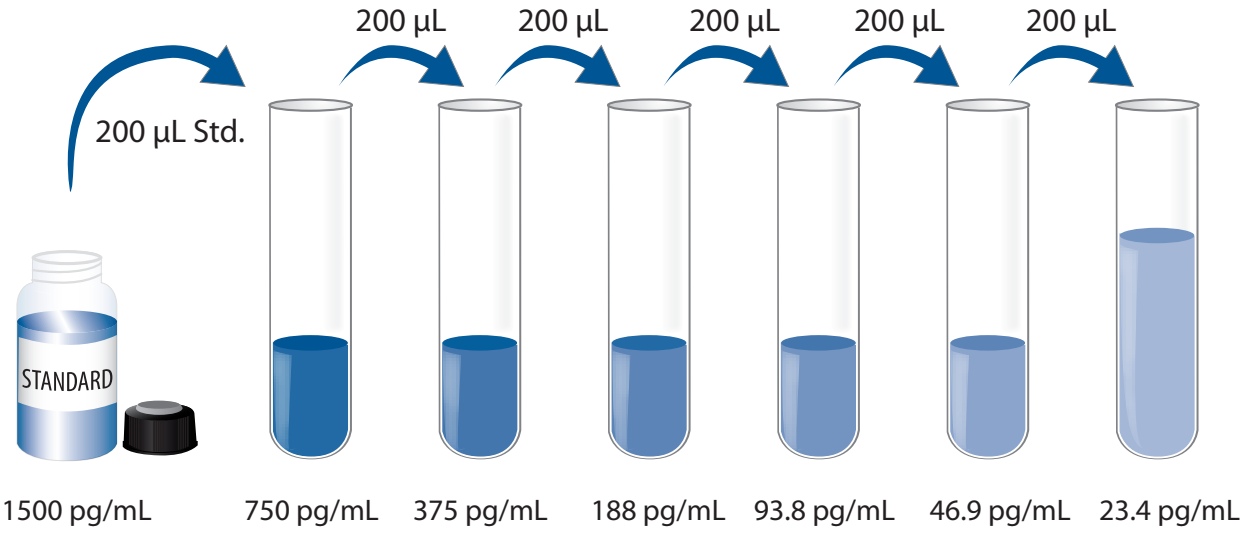
Mouse PlGF-2 Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

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. 100 µL of the resultant mixture is required per well.

Mouse PlGF-2 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse PlGF-2 Standard with Calibrator Diluent RD5-17. Do not substitute other diluents. This reconstitution produces a stock solution of 1500 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-17 into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse PlGF-2 Standard (1500 pg/mL) serves as the high standard. Calibrator Diluent RD5-17 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, Control, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, Control, 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 50 µL of Assay Diluent RD1-47 to each well.
4. Add 50 µL of Standard, Control, or sample* per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five 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 100 µL of Mouse PlGF-2 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 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. 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.

CALCULATION OF RESULTS

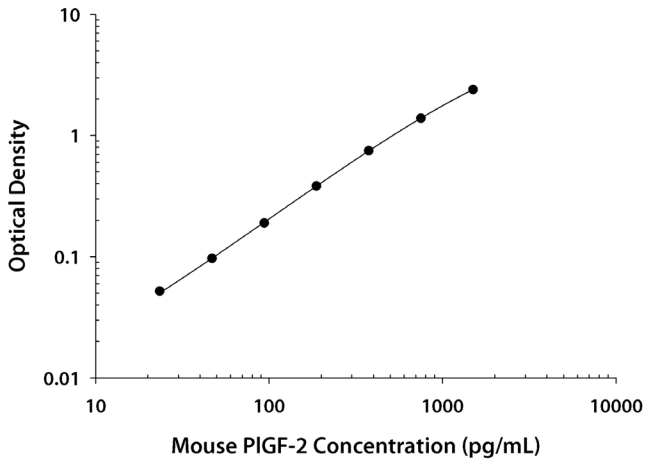
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse PlGF-2 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	0.034 0.034	0.034	—
23.4	0.083 0.088	0.086	0.052
46.9	0.128 0.134	0.131	0.097
93.8	0.221 0.228	0.224	0.190
188	0.415 0.419	0.417	0.383
375	0.763 0.800	0.782	0.748
750	1.351 1.495	1.423	1.389
1500	2.357 2.500	2.428	2.394

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	58.0	136	470	63.0	155	493
Standard deviation	4.6	7.6	14	6.0	15	43
CV (%)	7.9	5.6	3.0	9.5	9.7	8.7

RECOVERY

The recovery of mouse PlGF-2 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=6)	97	84-119%
Serum* (n=6)	90	81-102%
EDTA plasma* (n=6)	94	88-104%

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

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse PlGF-2 in each matrix were diluted with Calibrator Diluent and then assayed.

		Cell culture supernates (n=6)	Serum* (n=6)	EDTA plasma* (n=6)
1:2	Average % of Expected	102	102	99
	Range (%)	94-114	92-111	91-105
1:4	Average % of Expected	102	103	103
	Range (%)	93-112	94-115	95-110
1:8	Average % of Expected	103	105	104
	Range (%)	98-108	96-116	98-113
1:16	Average % of Expected	103	108	107
	Range (%)	93-110	99-118	99-118

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

SENSITIVITY

Two assays were evaluated and the minimum detectable dose (MDD) of mouse PlGF-2 ranged from 1.14-1.84 pg/mL. The mean MDD was 1.49 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.

CALIBRATION

This immunoassay is calibrated against a highly purified *Sf* 21-expressed recombinant mouse PlGF-2 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Twenty samples were evaluated for detectable levels of mouse PlGF-2 in this assay. All read lower than the lowest standard, 23.4 pg/mL. Serum samples from two pregnant mice (day 14) were evaluated for mouse PlGF-2 in this assay. The samples measured 39 pg/mL and 78 pg/mL.

Cell Culture Supernates:
Mouse splenocytes (1 x 10^6 cells/mL) were cultured for 3 days in RPMI supplemented with 10% fetal calf serum supplemented with 50 µM β-mercaptoethanol and 10 ng/mL of recombinant human IL-2. The cell culture supernate was assayed for mouse PlGF-2 and measured 295 pg/mL.

Two mouse lungs (1-2 mm pieces in 40 mL of medium) were cultured for 6 days in RPMI supplemented with 10% fetal calf serum. The cell culture supernate was assayed for mouse PlGF-2 and measured 3.6 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse PlGF-2.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the same factors at 50 ng/mL in a mid-range mouse PlGF-2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C10
Eotaxin
Flt-3 Ligand
G-CSF
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-10 R
IL-12 p40
IL-12 p70
IL-13
IL-17
IL-18
JE/MCP-1
KC
Leptin
LIF
MARC
MCP-5
MIP-1α
MIP-2
OSM
RANK Ligand
RANTES
TNF-α
TNF RI
TNF RII
Tpo
TARC
VEGF
VEGF R2
VEGF R3

Recombinant human:

PlGF
VEGF

High concentrations of soluble receptors to PlGF-2 can interfere with the measurement of mouse PlGF-2 in this assay.

Interference from added recombinant mouse VEGF R1/Fc Chimera was evaluated at different mouse PlGF-2 levels (54-438 pg/mL). The percentage of expected mouse PlGF-2 is listed below.

rmVEGF R1/Fc Chimera (ng/mL)	% of mPlGF-2 Expected
0	100
1	93
2	89
3	82
25	22

The concentration of soluble VEGF R1 in individual normal mouse serum and EDTA plasma samples was measured using a Mouse VEGF R1 Immunoassay (R&D Systems, Catalog # MVR100). The VEGF R1 values ranged from 0.9-4.0 ng/mL.

Sample Type	Average (ng/mL)	Range (ng/mL)
Serum (n=18)	1.8	0.9-3.5
EDTA plasma (n=20)	2.3	1.4-4.0

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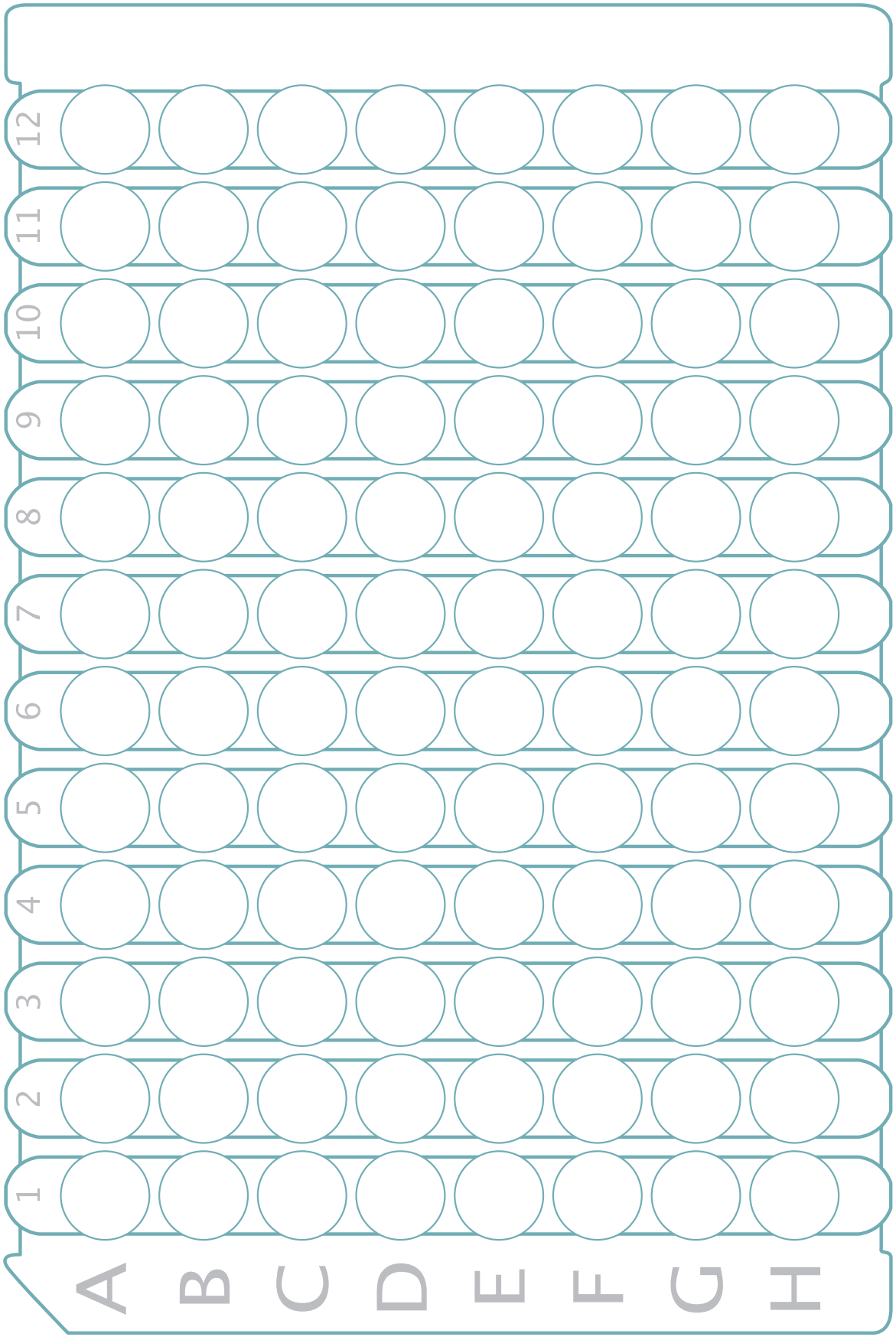
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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