Quantikine™ ELISA

Human PDGF-DD Immunoassay

Catalog Number DDD00

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

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION PAGE

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INTRODUCTION

The platelet-derived growth factor (PDGF) family consists of four differentially expressed, disulfide-linked homodimers (PDGF-AA, -BB, -CC, and -DD) and one heterodimer (PDGF-AB). These proteins regulate diverse cellular functions through interactions with PDGF Rα and Rβ (1, 2). Human PDGF-D shares 87% amino acid (aa) sequence identity with mouse and rat PDGF-D. The cystine knot PDGF/VEGF domain of PDGF-D shares 27-35% aa sequence identity with the corresponding regions of other PDGF family members, but only PDGF-C and –D contain a CUB domain and a hinge region with a basic cleavage site (1-4). Like PDGF-C, PDGF-D is secreted as a latent homodimer (100 kDa) which is activated by uPA (urokinase plasminogen activator) proteolysis to release a bioactive homodimer (35 kDa) containing the PDGF/VEGF homology domains (1-10). An inactive splice variant missing part of the PDGF/VEGF domain has been detected in mice, but not in humans (1, 11). PDGF-DD is widely expressed in embryonic and adult tissues and is active in the development of the kidney, eye and brain (1, 3, 10, 12-14). PDGF-DD is over-expressed in, and contributes to, disease states that include renal and hepatic fibrosis, pathological angiogenesis, mesangial proliferative glomerulopathy, IgA nephropathy, pulmonary lymphoid infiltration, post-infarction myocardial remodeling, and many cancers (1, 6-8, 14-24). PDGF-DD functions in autocrine, paracrine, and possibly endocrine manners (5, 6, 9, 21, 22).

PDGF-DD and its receptor, PDGF Rβ, are expressed in generally complementary patterns (10, 15, 16). Mature PDGF-DD associates with PDGF Rβ and primarily triggers signaling through PDGF Rβ homodimers rather than PDGF Rα/β heterodimers (1-4, 6, 7, 25). Through its receptor signaling, PDGF-DD functions as a chemoattractant for macrophages and a growth factor for cells such as vascular smooth muscle cells, lens epithelia, and many cancer cells (7, 8, 10, 11-18, 25-27). It has many pro-cancer activities, such as promoting epithelial-mesenchymal transition, inhibiting cancer cell apoptosis and enhancing cancer cell growth, migration, invasion and production of MMP-2, MMP-9, and VEGF (7, 13, 17-19, 20, 26, 27). Down-regulation of PDGF-DD expression or signaling inhibits cancer cell growth and angiogenesis (17).

The Quantikine™ Human PDGF-DD Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human PDGF-DD in cell culture supernates, serum, platelet-poor plasma, saliva, and human milk. It contains NS0-expressed recombinant human PDGF-DD and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human PDGF-DD 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 natural human PDGF-DD.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human PDGF-DD has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any PDGF-DD present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human PDGF-DD 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-DD 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 calibrator diluent and repeat the assay.
- Any variation in 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 PDGF-DD Microplate	894127	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human PDGF-DD.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human PDGF-DD Conjugate	894128	21 mL of a monoclonal antibody specific for human PDGF-DD conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human PDGF-DD Standard	894129	Recombinant human PDGF-DD in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-72	895367	11 mL of a buffered protein solution with preservatives.	
Calibrator Diluent RD6P	895118	21 mL of animal serum with preservatives. *Use diluted 1:2 in this assay. May contain a precipitate. Mix well before and during use.*	
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
- 50 mL and 500 mL graduated cylinders
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- **Polypropylene** test tubes for dilution of standards and samples
- Human PDGF-DD Controls (optional; R&D Systems®, Catalog # QC140)

PRECAUTIONS

PDGF-DD 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 SDS 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 ≤ -70 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Human Milk - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and centrifuge twice more for a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Use polypropylene tubes.

Serum samples require at least a 5-fold dilution. A suggested 5-fold dilution is 40 µL of sample + 160 µL of Calibrator Diluent RD6P (diluted 1:2)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *PDGF-DD is detectable in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

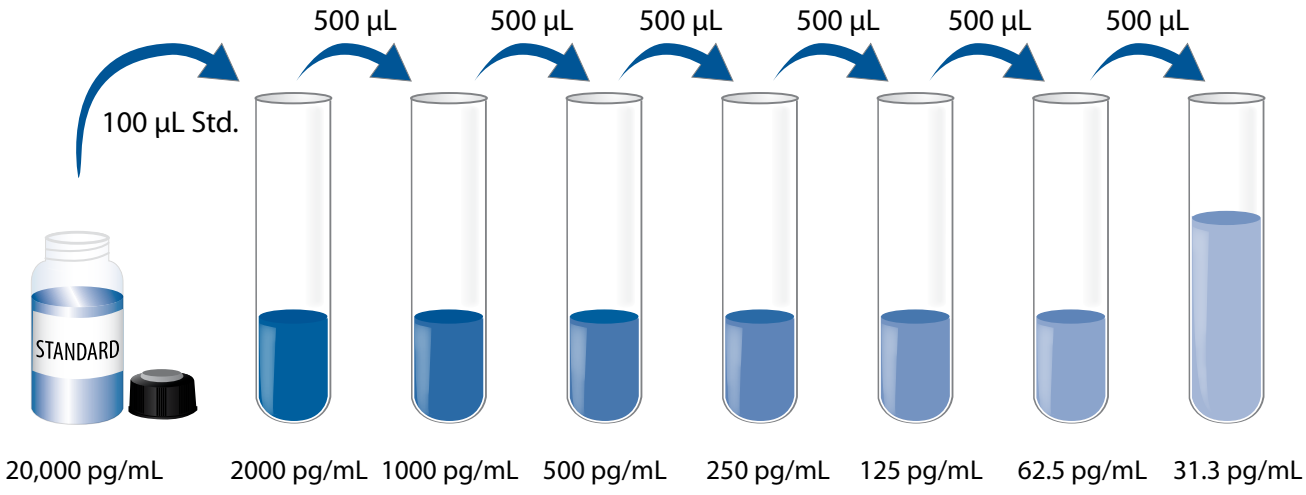
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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 RD6P (diluted 1:2) - Add 10 mL of Calibrator Diluent RD6P to 10 mL of deionized or distilled water to prepare 20 mL of Calibrator Diluent RD6P (diluted 1:2). Prepare fresh for each use. If a full plate is not being assayed, adjust volumes accordingly.

Human PDGF-DD Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human PDGF-DD Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD6P (diluted 1:2) into the 2000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. Calibrator Diluent RD6P (diluted 1:2) 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, controls, and samples be assayed in duplicate.

Note: *PDGF-DD is detectable in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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 RD1-72 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 on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human PDGF-DD Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

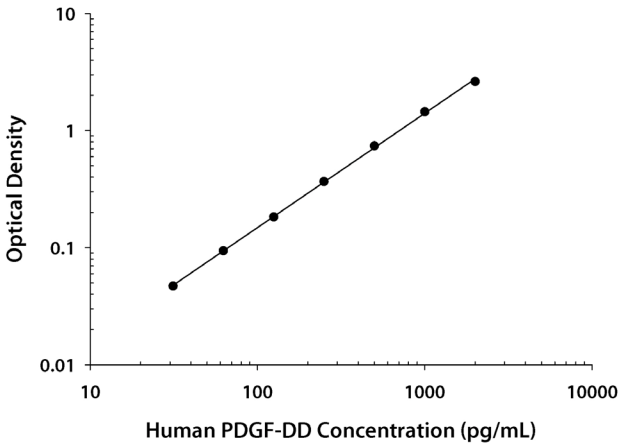
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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-DD 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

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	0.009 0.009	0.009	—
31.3	0.055 0.056	0.056	0.047
62.5	0.102 0.104	0.103	0.094
125	0.191 0.192	0.192	0.183
250	0.374 0.377	0.376	0.367
500	0.742 0.755	0.749	0.740
1000	1.452 1.461	1.457	1.448
2000	2.596 2.668	2.632	2.623

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)	176	510	986	198	559	1095
Standard deviation	3.69	11.9	17.2	16.0	38.5	55.3
CV (%)	2.1	2.3	1.7	8.1	6.9	5.1

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	102	83-112%
Serum* (n=4)	101	86-112%
Platelet-poor EDTA plasma (n=4)	93	83-109%
Platelet-poor heparin plasma (n=4)	94	84-113%

*Samples were diluted prior to assay.

LINEARITY

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

		Cell culture media (n=4)	Serum* (n=4)	Platelet-poor EDTA plasma (n=4)	Platelet-poor Heparin plasma (n=4)	Human milk (n=4)
1:2	Average % of Expected	103	102	105	103	99
	Range (%)	100-104	100-105	101-106	99-110	91-111
1:4	Average % of Expected	103	102	97	106	98
	Range (%)	101-105	99-108	85-105	101-112	90-108
1:8	Average % of Expected	102	103	100	106	99
	Range (%)	100-103	100-107	85-109	101-112	91-111
1:16	Average % of Expected	99	98	95	102	95
	Range (%)	97-101	94-103	80-103	97-110	87-107

*Samples were diluted prior to assay.

SENSITIVITY

Twenty-four assays were evaluated and the minimum detectable dose (MDD) of human PDGF-DD ranged from 0.699-3.51 pg/mL. The mean MDD was 1.67 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 NS0-expressed recombinant human PDGF-DD produced at R&D Systems®.

SAMPLE VALUES

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

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=31)	2519	100	1430-4106
Platelet-poor EDTA plasma (n=31)	34.2	9.7	ND-39.3
Platelet-poor heparin plasma (n=31)	68.0	84	ND-184
Human milk (n=15)	875	87	ND-3274

ND=Non-detectable

Nine saliva samples were evaluated for the presence of human PDGF-DD in this assay. One sample measured 48.4 pg/mL. The remaining eight samples measured less than the lowest standard, 31.3 pg/mL.

Cell Culture Supernates:
A549 human lung carcinoma cells were cultured in Kaighn's F-12 and supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 100 U/mL penicillin until confluent. An aliquot of the cell culture supernate was removed, assayed for human PDGF-DD, and measured 74.5 pg/mL.

THP-1 human acute monocytic leukemia cells were cultured in RPMI 1640 and supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol, and 2 mM L-glutamine for one week. Cells were then stimulated with 100 ng/mL LPS for 1 day. An aliquot of the cell culture supernate was removed, assayed for human PDGF-DD, and measured 57.6 pg/mL.

IMR-90 human lung fibroblasts were cultured in MEM and supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until confluent. An aliquot of the cell culture supernate was removed, assayed for human PDGF-DD, and measured 133 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human PDGF-DD.

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 recombinant human PDGF-DD control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

EGF
FGF acidic
FGF basic
FGF-4
FGF-5
FGF-6
KGF/FGF-7
FGF-9
FGF-10
FGF-18
Flt-3 Ligand
G-CSF
GM-CSF
HB-EGF
HGF
IGF-I
IGF-II
M-CSF
MSP/MST1
MSP β Chain
Neuregulin-1/NRG1
β-NGF
NRG1-β/HRG1-β
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
PDGF-CC
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 Ligand
G-CSF
GM-CSF
M-CSF
PDGF-CC
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
porcine PDGF

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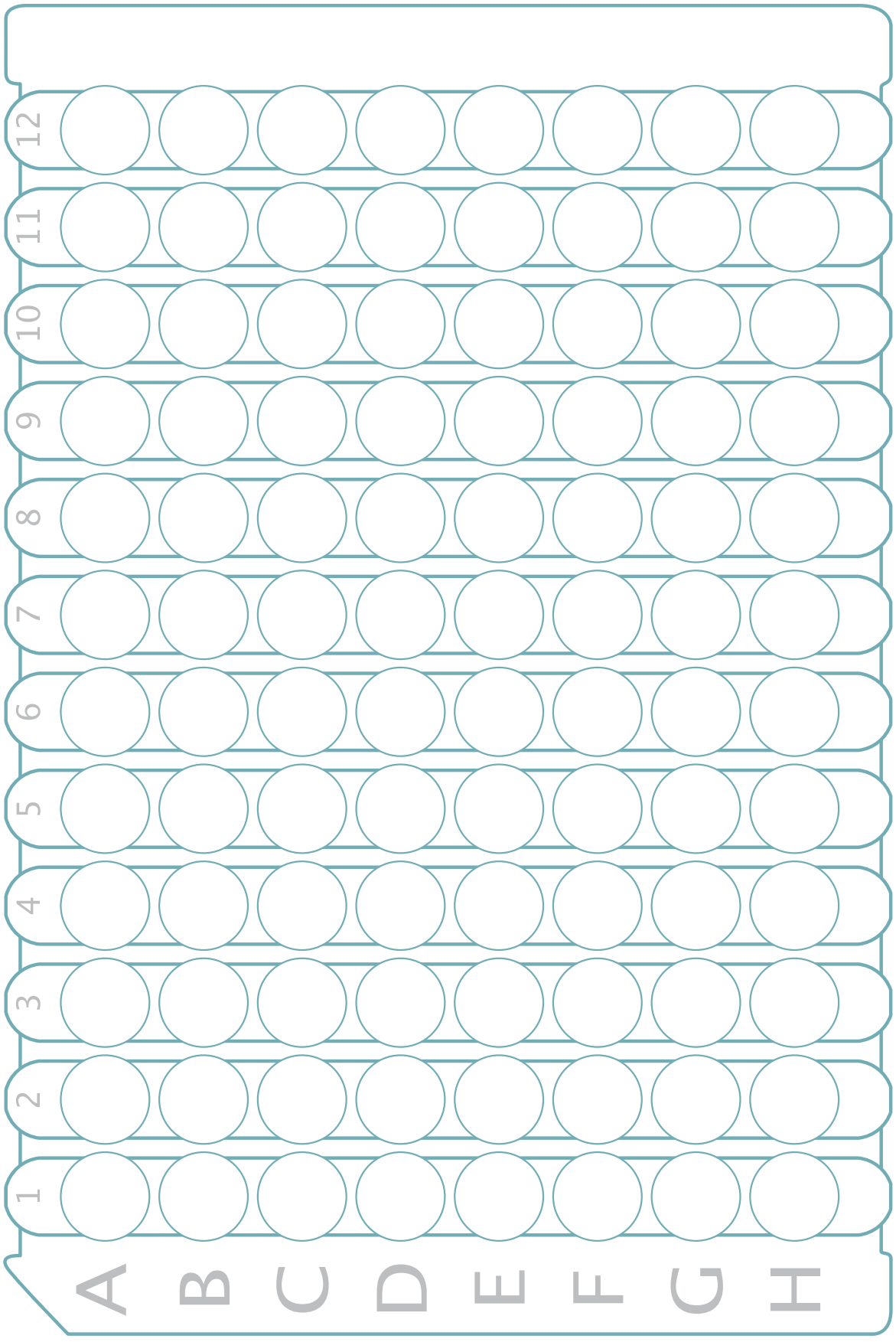
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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