Quantikine® ELISA

Human DLL1 Immunoassay

Catalog Number DLL10

For the quantitative determination of Delta-like Protein 1 (DLL1) concentrations in cell culture supernates, serum, plasma, urine, 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.

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INTRODUCTION

Delta-like protein 1 (DLL1) is a 90-100 kDa type I transmembrane glycoprotein in the Delta/Serrate/Lag-2 (DSL) family of Notch ligands (1, 2). It plays an important role in the morphogenesis and remodeling of many developing and adult tissues. DLL1 is over expressed in a number of cancers and contributes to tumorigenesis (3-5). The extracellular domain (ECD) of human DLL1 contains one DSL domain and eight EGF-like repeats (3). It shares 91% amino acid (aa) sequence identity with mouse and rat DLL1 and 26%, 37%, and 54% aa sequence identity with human DLL2, 3, and 4, respectively. A 60 kDa ECD fragment of DLL1 can be shed from the cell surface by ADAM9, 10, 12, 17, or MMP-14 mediated proteolysis (6-9). Removal of the DLL1 ECD by ADAM12 cleavage *in cis* increases the Notch responsiveness of that cell, suggesting that coexpressed DLL1 is inhibitory to Notch signaling (6). In contrast, DLL1 shedding on neighboring cells can reduce Notch signaling (7). The residual membrane-bound portion of DLL1 is cleaved by γ-secretase to release the approximately 30-40 kDa intracellular domain which translocates to the nucleus and regulates gene transcription (10-12).

DLL1 exerts its biological activity through receptors in the Notch family. These four receptors also mediate the responses to other canonical Notch ligands including DLL3, DLL4, Jagged-1, and Jagged-2. Non-canonical ligands (*e.g.* Contactin-1, Contactin-2, DNER, MAGP-1, MAGP-2, NOV, and Pref-1) are structurally dissimilar to DLL1 but can also modulate Notch activity (1, 2). Notch-1 is a 300 kDa type I transmembrane glycoprotein that contains 36 EGF-like repeats and three Lin-12/notch repeats (LNR) in its ECD (13, 14). Notch-1 undergoes intracellular furin-type proteolytic cleavage, forming a noncovalent heterodimer of the 200 kDa ligand-binding extracellular region with the 120 kDa transmembrane/cytoplasmic portion (15, 16). Upon ligand binding, the Notch ECD is shed by TACE/ADAM17 or ADAM10 mediated cleavage (9, 17, 18). Intramembrane cleavage by γ-secretase releases the Notch intracellular domain (NICD) which translocates to the nucleus, activating transcription of Notch-responsive genes (19, 20).

DLL1 expression is developmentally regulated and plays a role in central nervous system morphogenesis (21), intervertebral joint formation (22), and inner ear hair cell development and patterning (23). It regulates the homeostasis and differentiation of neural (24, 25), pancreatic (26), and myogenic (7, 27) progenitor cells. DLL1 promotes the Notch-dependent proliferation of hematopoietic progenitor cells (28, 29) as well as T cell lineage commitment and differentiation (29-31). It inhibits commitment to the B cell lineage but is required for the differentiation of already-committed B cells to plasma cells and marginal zone B cells (30-32). DLL1 expression is upregulated on arterial vascular endothelial and perivascular cells in response to angiogenic factors (*e.g.* VEGF, FGF-2) or injury (33, 34). Its activation of Notch contributes to arteriogenesis by inducing the upregulation of VEGF R and Ephrin-B2 (33, 35). DLL1 is upregulated on macrophages during influenza virus infection and contributes to antiviral immunity (36).

The Quantikine Human DLL1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human DLL1 in cell culture supernates, serum, plasma, urine, and human milk. It contains NS0-expressed recombinant human DLL1 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human DLL1 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 human DLL1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for DLL1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any DLL1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for DLL1 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 DLL1 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 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 soluble receptors, binding proteins, and 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
DLL1 Microplate	894146	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against DLL1.	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.*
DLL1 Conjugate	894147	21 mL of monoclonal antibody against DLL1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
DLL1 Standard	894148	50 ng of recombinant human DLL1 in a buffered protein base with preservatives; lyophilized.	
Assay Diluent RD1-19	895467	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-5	895485	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives.	
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.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human DLL1 Controls (optional; available from R&D Systems).

PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

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 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 heparin or EDTA 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: *Citrate plasma has not been validated for use in this assay.*

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

Human Milk - Centrifuge for 15 minutes at 10,000 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

Serum and plasma samples require at least a 5-fold dilution. A suggested 5-fold dilution is 50 µL of sample + 200 µL of Calibrator Diluent RD5-5.

REAGENT PREPARATION

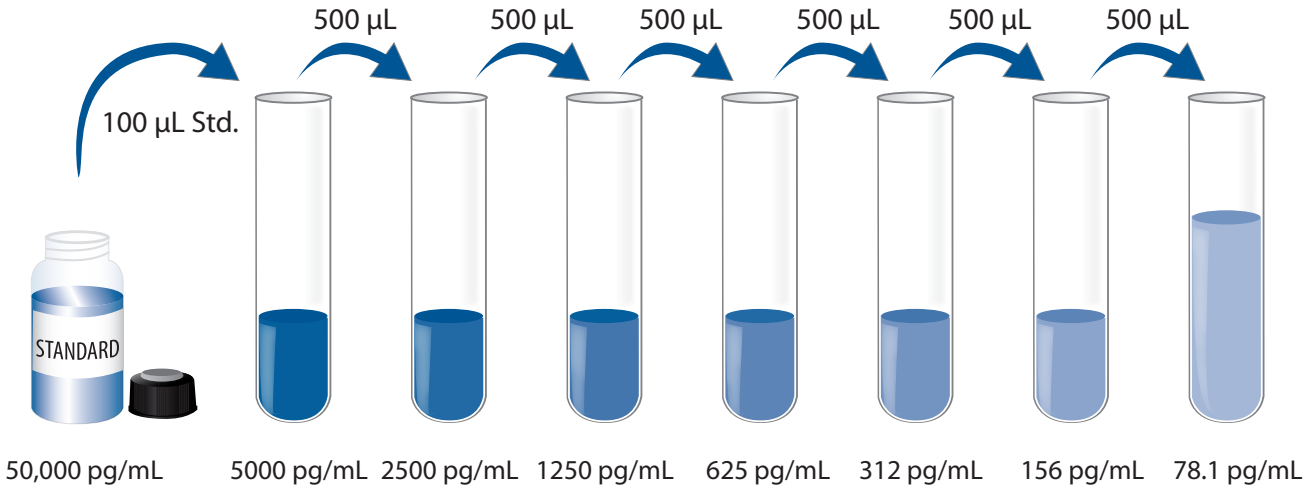
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into 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.

DLL1 Standard - Reconstitute the DLL1 Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 50,000 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.

Pipette 900 µL of Calibrator Diluent RD5-5 into the 5000 pg/mL tube. Pipette 500 µL of Calibrator Diluent RD5-5 into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-5 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, 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 RD1-19 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 DLL1 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

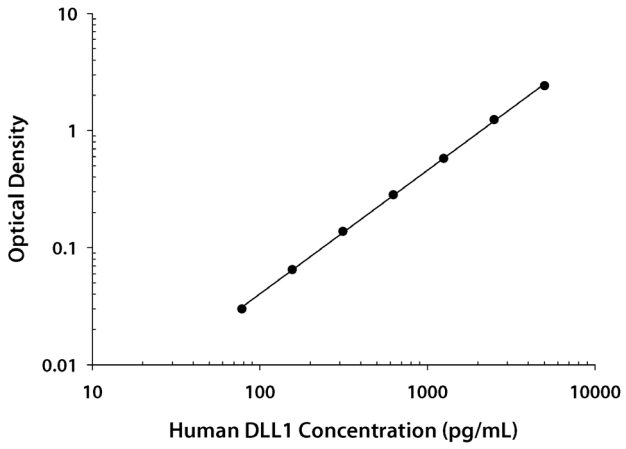
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

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 DLL1 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	—
78.1	0.038 0.039	0.039	0.030
156	0.072 0.076	0.074	0.065
312	0.146 0.147	0.147	0.138
625	0.290 0.294	0.292	0.283
1250	0.579 0.592	0.586	0.577
2500	1.232 1.260	1.246	1.237
5000	2.425 2.430	2.428	2.419

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	638	1468	2934	592	1340	2714
Standard deviation	16.0	38.1	76.1	42.4	85.7	189
CV (%)	2.5	2.6	2.6	7.2	6.4	7.0

RECOVERY

The recovery of DLL1 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	102	96-108%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of DLL1 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)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine (n=4)	Human milk (n=4)
1:2	Average % of Expected	102	103	104	105	105	106
	Range (%)	95-107	99-107	102-106	102-107	102-108	102-113
1:4	Average % of Expected	102	104	105	106	102	103
	Range (%)	95-109	100-107	104-107	103-108	100-104	98-110
1:8	Average % of Expected	102	104	107	108	———	100
	Range (%)	97-104	100-107	105-109	104-110	———	94-108
1:16	Average % of Expected	99	104	106	106	———	———
	Range (%)	96-103	101-109	104-110	103-110	———	———

*Samples were diluted prior to assay.

SENSITIVITY

Twenty-seven assays were evaluated and the minimum detectable dose (MDD) of DLL1 ranged from 1.94-17.4 pg/mL. The mean MDD was 5.52 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 NS0-expressed recombinant human DLL1 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Urine/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of DLL1 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=36)	8054	5792-11,311	1350
EDTA plasma (n=36)	8181	5711-11,343	1401
Heparin plasma (n=36)	8269	5744-11,261	1459
Human milk (n=9)	705	275-1560	369

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Urine (n=10)	307	90	ND-543

ND=Non-detectable

Cell Culture Supernates:
MCF-7 human breast cancer cells were cultured in DMEM/Kaughn's F-12 (50/50) and supplemented with 10% fetal bovine serum, and 2 mM L-glutamine. An aliquot of the cell culture supernate was removed, assayed for levels of natural human DLL1, and measured 366 pg/mL.

ME-180 human cervical epithelial carcinoma cells were cultured in McCoy's 5a and supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for levels of natural human DLL1, and measured 406 pg/mL.

CHP-100 human neuroblastoma cells were cultured in RPMI and supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% antibiotics, and 0.25 mL gentamicin. An aliquot of the cell culture supernate was removed, assayed for levels of natural human DLL1, and measured 156 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human DLL1.

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 DLL1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Adiponectin
Angiopoietin-like 3
Clusterin
DLL3
DLL4
DNER
FABP4
FTO
Glutathione Peroxidase 3
IL-33
Jagged 1
Leptin
Notch-1
Notch-2
Notch-3
RBP4
Resistin
Serpin A12

Recombinant mouse:

Adiponectin
DLL1
DLL4

Recombinant rat:

DLL1
Serpin A12

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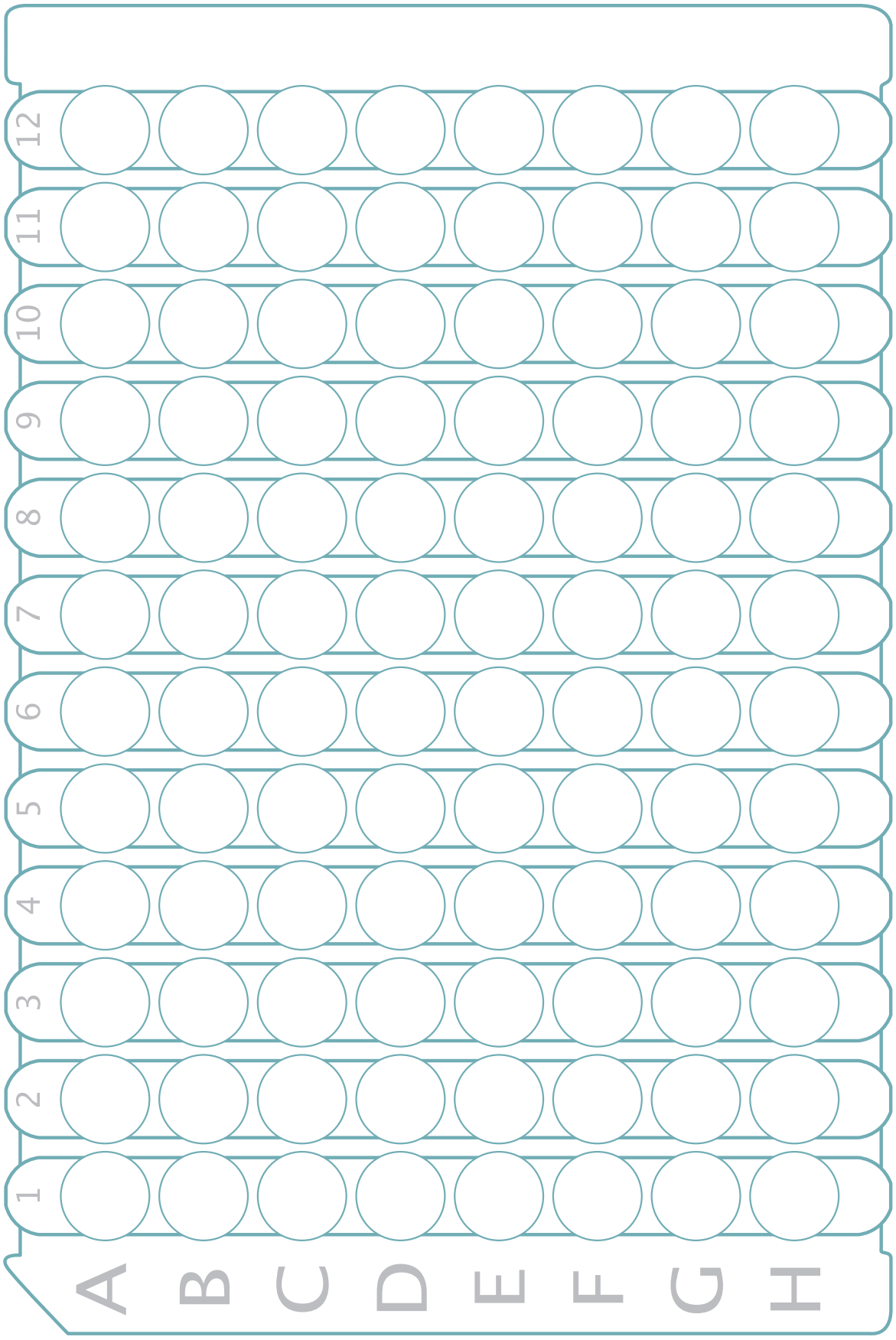
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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