Quantikine® ELISA

Mouse Endoglin/CD105 Immunoassay

Catalog Number MNDG00

For the quantitative determination of mouse Endoglin concentrations in cell culture supernates, cell lysates, 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

Endoglin (CD105) is a 90 kDa type I transmembrane glycoprotein of the zona pellucida (ZP) family of proteins (1-6). Endoglin and TGF-β RIII/betaglycan are type III receptors for TGF-β superfamily ligands, sharing 71% amino acid (aa) sequence identity within the transmembrane (TM) and cytoplasmic domains (1). Mouse Endoglin cDNA encodes 653 amino acids including a 26 aa signal sequence, a 555 aa extracellular domain (ECD) with a two-part ZP domain, a TM domain, and a 47 aa cytoplasmic domain (1-3). A mouse isoform with a 35 aa cytoplasmic domain (S-Endoglin) can oppose effects of long (L) Endoglin (7-9). Endoglin is highly expressed on proliferating vascular endothelial cells, chondrocytes, and syncytiotrophoblasts of term placenta, with lower amounts on hematopoietic, mesenchymal and neural crest stem cells, activated monocytes, and lymphoid and myeloid leukemic cells (1-6, 10-15). Endoglin expression can be increased by TGF-β, senescence (S-Endoglin in endothelium), stress-induced S100B (in placenta or amnion), or hypoxia (6, 7, 16, 17). The mouse Endoglin ECD shares 69%, 84%, 62%, 63%, and 66% aa sequence identity with human, rat, bovine, porcine, and canine Endoglin, respectively.

In complexes with type II receptors (TGF-β RII), Endoglin homodimers can interact with TGF-β1 and TGF-β3 but not TGF-β2 (18, 19). Similarly, they interact with activin A and BMP-7 via activin type IIA or B receptors, and with BMP-2 via BMPR-IA/ALK-3 or BMPR-IB/ALK-6 (19). BMP-9, however, is reported to bind Endoglin directly (3, 20). Endoglin modification of ligand-induced signaling can vary according to conditions. For example, expression of Endoglin can inhibit TGF-β1 signals but enhance BMP-7 signals in the same myoblast cell line (21, 22). Many studies find that Endoglin-deficient endothelial cells show inhibited signaling in response to TGF-β (3-6, 20, 23).

Deletion of mouse Endoglin causes lethal vascular and cardiovascular defects (24). Human Endoglin haplo-insufficiency, which reduces both transmembrane and soluble Endoglin, can cause the vascular disorder hereditary hemorrhagic telangiectasia type I (3-5, 25). These abnormalities confirm the essential function of Endoglin in differentiation of vascular smooth muscle, angiogenesis, and neovascularization (2-5, 10, 23-25). Myocardial infarction is associated with low circulating Endoglin, with lowest values correlating with poor prognosis (26). In pre-eclampsia, high levels of proteolytically generated soluble Endoglin and VEGF R1 (sFlt-1), along with low placenta growth factor (PlGF), are pathogenic due to anti-angiogenic activity (27, 28). The elevated soluble Endoglin can be detected in either blood or urine (27-30). Soluble Endoglin can also be elevated in knee osteoarthritis (both in plasma and synovial fluid), sickle cell disease, severe malaria, and metastatic colorectal cancer (31-34). Endoglin has been proposed as a therapeutic target for cancer due to its high expression in tumor vasculature (6, 15).

The Quantikine Mouse Endoglin Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse Endoglin in cell culture supernates, cell lysates, serum, plasma, and urine. It contains NS0-expressed recombinant mouse Endoglin and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant factor accurately. Results obtained using natural Endoglin showed dose-response curves that were parallel to the standard curves obtained using the recombinant kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse Endoglin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse Endoglin has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any Endoglin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse Endoglin 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 Endoglin 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.
- For best results, pipette reagents and samples into the center of each well.
- 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 Endoglin Microplate	893863	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse Endoglin.	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 Endoglin Conjugate	893864	12 mL of a polyclonal antibody specific for mouse Endoglin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse Endoglin Standard	893865	20 ng of recombinant mouse Endoglin in a buffered protein base with preservatives; lyophilized.	
Mouse Endoglin Control	893866	Recombinant mouse Endoglin in a buffered protein base with preservatives; lyophilized. The concentration range of mouse Endoglin after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.	
Assay Diluent RD1S	895137	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-4	895435	2 vials (21 mL/vial) of a buffered protein base 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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.

If using cell lysates, the following supplies are also required:

- Cell Lysis Buffer 2 (R&D Systems, Catalog # 895347).
- Phosphate-buffered saline (PBS)

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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Cell Lysates - Cell must be lysed prior to assay as directed in the Sample Values section.

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 samples are not suitable for use in this assay.

Urine - Collect urine using a metabolic cage. Remove any particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional particulates that may appear after storage.

SAMPLE PREPARATION

Serum and plasma samples require a 4-fold dilution. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent RD5-4.

Urine samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD5-4.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

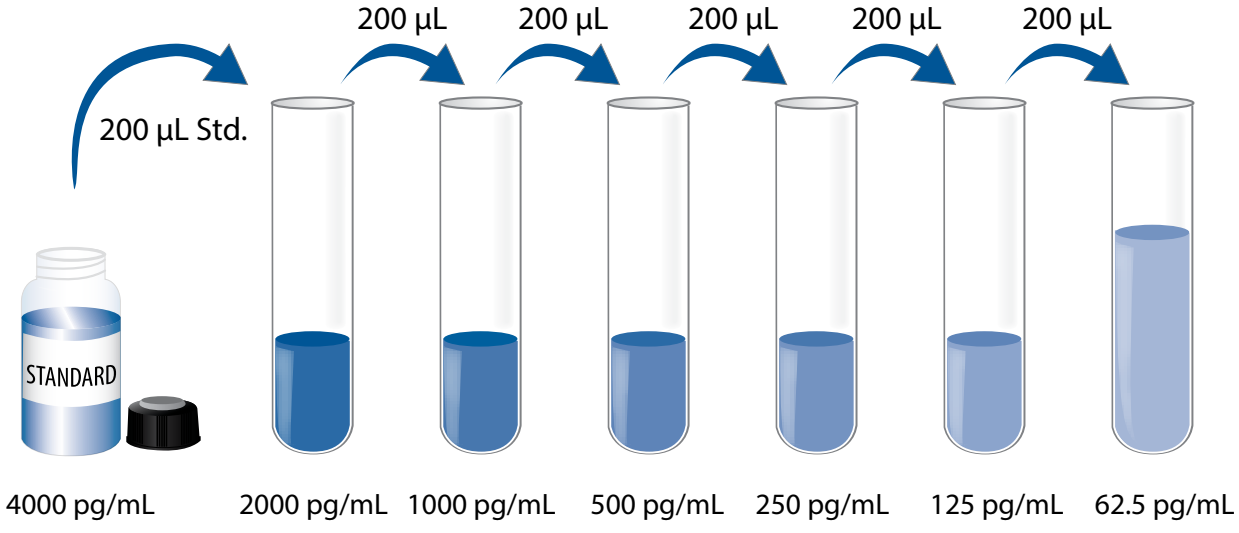
Mouse Endoglin Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

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 Endoglin Standard - Reconstitute the Mouse Endoglin Standard with 5.0 mL of Calibrator Diluent RD5-4. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-4 into each of six tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse Endoglin Standard (4000 pg/mL) serves as the high standard. Calibrator Diluent RD5-4 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, standards, Control, and samples as directed by 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 RD1S 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 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 Endoglin Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours on the shaker.
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 **on the benchtop. 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 the Sample Preparation section.

CALCULATION OF RESULTS

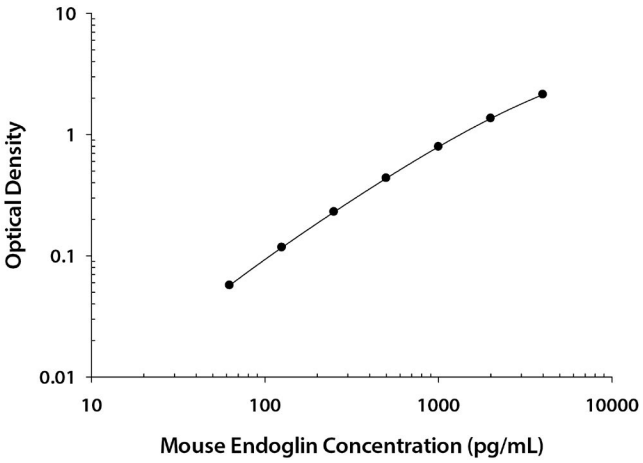
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 Endoglin 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	0.018 0.019	0.018	—
62.5	0.074 0.077	0.075	0.057
125	0.130 0.140	0.135	0.117
250	0.245 0.251	0.248	0.230
500	0.448 0.460	0.454	0.436
1000	0.786 0.836	0.811	0.793
2000	1.368 1.384	1.376	1.358
4000	2.129 2.177	2.153	2.135

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 thirty-two separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	32	32	32
Mean (pg/mL)	182	463	1472	176	552	1457
Standard deviation	9.4	22.0	40.2	15.3	36.3	71.4
CV (%)	5.2	4.8	2.7	8.7	6.6	4.9

RECOVERY

The recovery of mouse Endoglin levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture samples (n=7)	102	93-114%
Cell lysates (n=1)	115	——
Serum* (n=4)	107	100-117%
EDTA plasma* (n=4)	107	99-114%
Heparin plasma* (n=4)	109	108-111%
Urine* (n=4)	107	95-118%

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

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse Endoglin were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Cell lysates (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine* (n=4)
1:2	Average % of Expected	99	98	101	100	99	94
	Range (%)	89-105	94-101	98-102	98-102	97-101	93-97
1:4	Average % of Expected	99	98	99	98	100	92
	Range (%)	87-106	92-103	92-103	95-99	94-109	87-99
1:8	Average % of Expected	99	98	96	96	102	96
	Range (%)	94-103	92-103	90-101	93-99	93-113	90-105
1:16	Average % of Expected	105	100	96	95	104	———
	Range (%)	97-119	96-105	90-103	91-100	95-114	———

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

SENSITIVITY

Seventy-four assays were evaluated and the minimum detectable dose (MDD) of mouse Endoglin ranged from 1.28-13.6 pg/mL. The mean MDD was 4.17 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 mouse Endoglin produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Urine - Samples were evaluated for the presence of mouse Endoglin in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=20)	4876	3160-9865	1780
EDTA plasma (n=20)	3620	2212-6558	1344
Heparin plasma (n=20)	5675	2963-8247	1750
Urine (n=20)	1666	147-3720	870

Cell Culture Supernates:
Organs from mice were removed, rinsed in PBS, kept on ice in tubes containing PBS, homogenized using a tissue homogenizer, and centrifuged to remove excess PBS. Homogenized cells were cultured in 100 mL of RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 24 hours. Aliquots of the cell culture supernates were removed and assayed for levels of mouse Endoglin.

Tissue Type	Observed Levels (pg/mL)
Brain	301
Heart	1628
Spleen	3840

L-929 mouse fibroblast cells, seeded at 0.5 x 10^5 cells/mL, were cultured in MEM (NEAA) supplemented with 10% equine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 3 days. An aliquot of the cell culture supernate was removed, assayed for levels of mouse Endoglin, and measured 373 pg/mL.

Cell Lysates - Organs from mice were removed, rinsed in PBS, kept on ice in tubes containing PBS, homogenized using a tissue homogenizer, and centrifuged to remove excess PBS. A 1:1 ratio of Cell Lysis Buffer 2 was added to the homogenized cells and lysed at room temperature for 30 minutes with gentle agitation. Cells were then centrifuged to remove debris. Aliquots from the cell lysates were removed and assayed for levels of mouse Endoglin.

Tissue Type	Observed Levels (pg/mL)
Brain	3430
Heart	5200
Spleen	7640

SPECIFICITY

This assay recognizes natural and recombinant mouse Endoglin.

The factors listed below were prepared at 500 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 500 ng/mL in a mid-range mouse Endoglin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

Activin RIIB
ALK-1
BMP-7
BMPR-IA
BMPR-IB
TGF-β RI
TGF-β RII

Recombinant human:

Activin
Endoglin
Latent TGF-β1
TGF-β1
TGF-β3
TGF-β RI
TGF-β RII
TGF-β RIII

Recombinant rat:

Endoglin

Natural protein:

bovine Collagen

Recombinant mouse BMP-9 and recombinant human (rh) TGF-β3 combined with rhTGF-β RI and rhTGF-β RII interfere in this assay.

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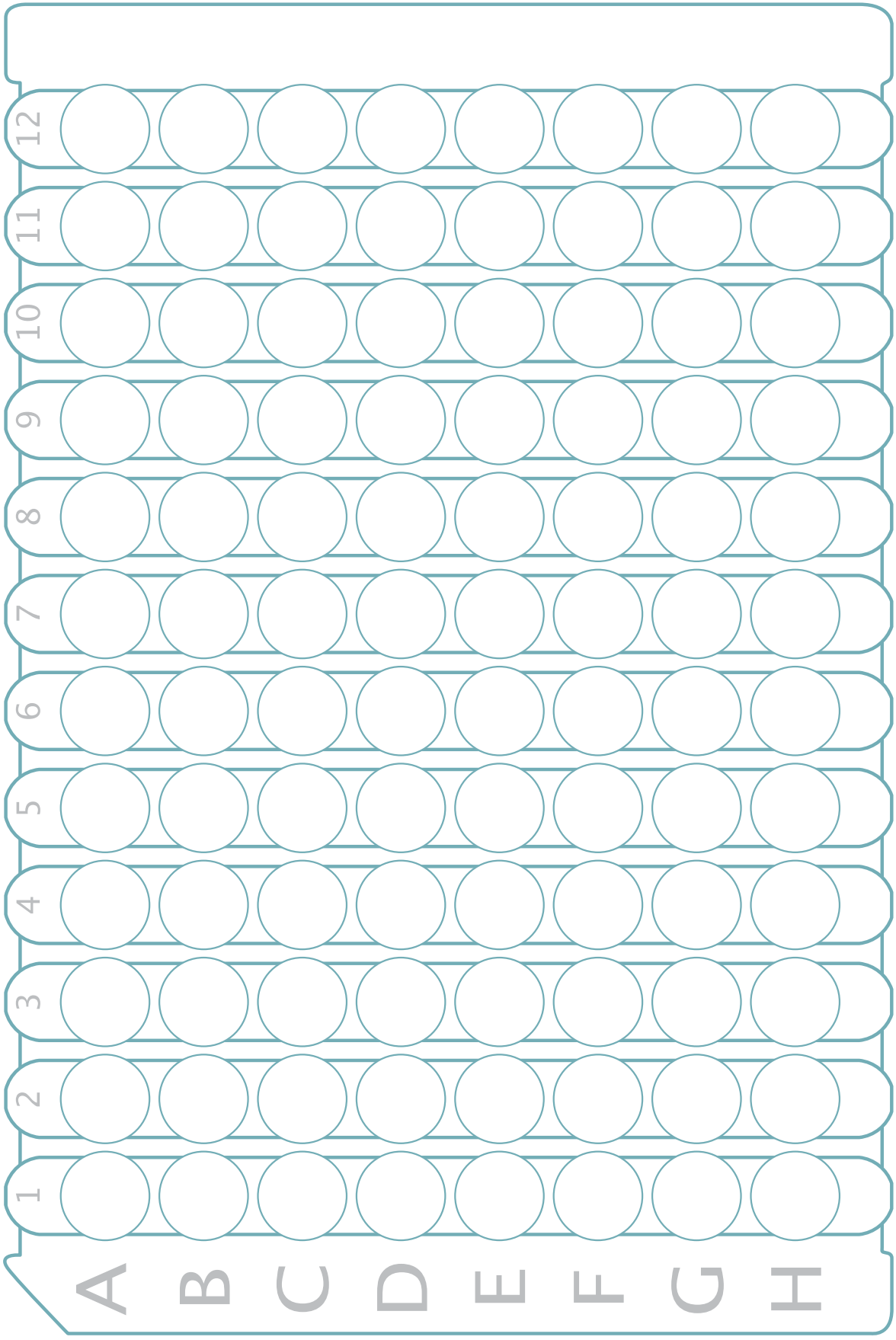
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES