Quantikine® ELISA

Mouse Tie-2 Immunoassay

Catalog Number MTE200

For the quantitative determination of mouse Tie-2 concentrations in cell culture supernates, tissue homogenates, serum, and plasma.

Note: The standard reconstitution method has changed. Read this package insert in its entirety before using this product.

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

Mouse Tie-2 is a 145 kDa, type I transmembrane glycoprotein that was originally identified during a search for receptors involved in mouse cardiogenesis (1). It is synthesized as an 1122 amino acid (aa) precursor that contains an 18 aa signal sequence, a 726 aa extracellular region, a 25 aa transmembrane segment, and a 353 aa cytoplasmic tail (2-4). The extracellular region has an unusual construct, being composed of two C2 Ig-like domains (80-90 aa in length), three EGF-like motifs (≈ 45 aa in length), and three fibronectin (FN)-type III repeats (≈ 90 aa in length) (4). The cytoplasmic region has a split tyrosine kinase domain with one 100 aa and a second 150 aa segment (2, 4). Tie-2 presumably functions as a ligand-induced homodimer (5, 6). Mouse Tie-1 has also been cloned (3). Demonstrating the same general architecture as Tie-2, it shows less than 50% aa identity to Tie-2 in the extracellular region (3). Mouse Tie-2 shares 90% and 89% aa sequence identity in the extracellular region to human and bovine Tie-2, respectively (3, 7). Human Tie-2 ligands are active on mouse Tie-2 positive cells (8-10). Cells known to express Tie-2 are few in number and include embryonic (1, 11) and adult endothelial cells (1, 12, 13), plus hematopoietic stem cells (8, 14, 15) and B cells (15). Tie-2 is known to exist as a soluble form, most likely the result of proteolytic cleavage (16, 17).

There are three mouse ligands for the Tie-2 receptor: the 68 kDa Angiopoietin-1 (Ang-1), the 70 kDa Ang-2, and the 72 kDa Ang-3 (9). The Angiopoietins are products of separate genes that share less than 65% aa sequence identity relative to one another (18-20). All of them form dimers and possibly multimers. Each molecule is characterized by the presence of an N-terminal coiled-coil quaternary structure that is used for dimerization, and a C-terminal fibronectin-like domain that is used for receptor binding (19, 21). It has been reported that Ang-1 is a Tie-2 activator, while Ang-2 and Ang-3 are Tie-2 antagonists (18-20). This appears to be only partially correct. All three Angiopoietins are activating, and the antagonist action of Ang-2 is a context, or cell-specific phenomenon (9, 21, 22).

The function of Tie-2 is complex, and apparently context and ligand dependent. In general, Tie-2 is said to be important for maintaining vascular integrity. It mediates endothelial cell-smooth muscle cell communication and inhibits endothelial cell apoptosis. It is also absolutely required for embryonic development of the endocardium (5, 23), and postnatal bone marrow hematopoiesis (24, 25).

The Quantikine® Mouse Tie-2 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse Tie-2 in cell culture supernates, tissue homogenates, serum, and plasma. It contains NS0-expressed recombinant mouse Tie-2 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse Tie-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 natural mouse Tie-2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. Monoclonal antibodies specific for mouse Tie-2 have been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any Tie-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse Tie-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 Tie-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 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.
- It is recommended that the samples be pipetted within 15 minutes.
- 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 Tie-2 Microplate	892672	96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibodies specific for mouse Tie-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 Tie-2 Standard	892674	Recombinant mouse Tie-2 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Mouse Tie-2 Control	892675	Recombinant mouse Tie-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 Tie-2 Conjugate	892673	12 mL of a polyclonal antibody specific for mouse Tie-2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1N	895488	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-17	895512	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 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.

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. 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.

Tissue Homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, spleen, lung, or liver tissue from one adult female mouse was rinsed with 1X PBS to remove excess blood, homogenized in 5-10 mL of 1X PBS and stored overnight at ≤ -20 °C. Homogenates were centrifuged for 5 minutes at 5000 x g.

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 may not be suitable for use in this assay.

SAMPLE PREPARATION

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

REAGENT PREPARATION

Bring all reagents to room temperature before use.

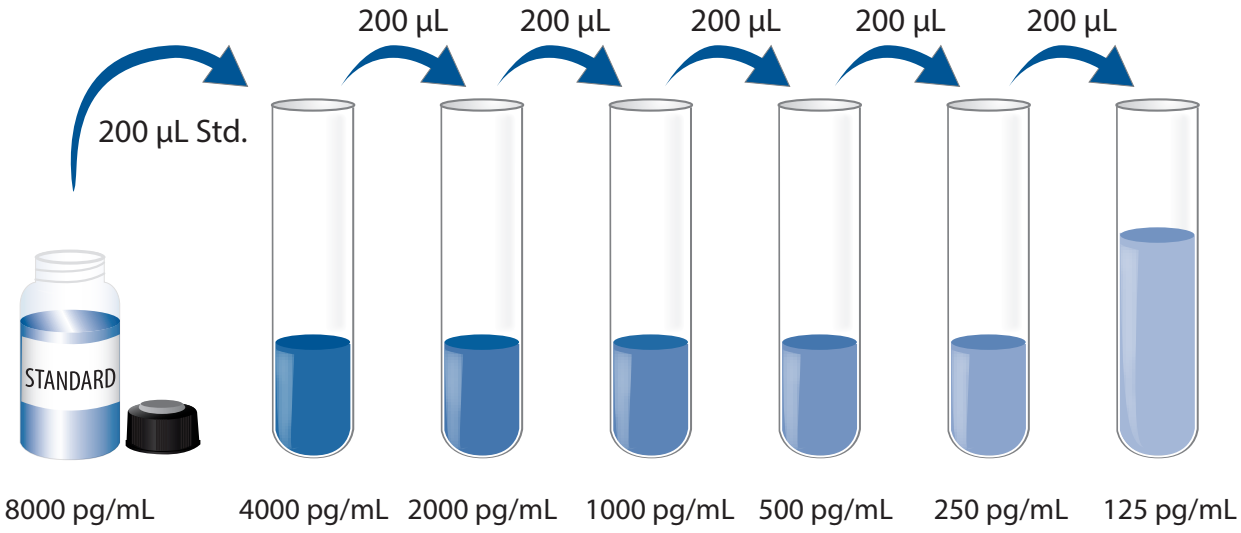
Mouse Tie-2 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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 Tie-2 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse Tie-2 Standard with Calibrator Diluent RD5-17. Do not substitute other diluents. This reconstitution produces a stock solution of 8000 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 RD5-17 into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse Tie-2 Standard (8000 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 RD1N 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 Tie-2 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 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

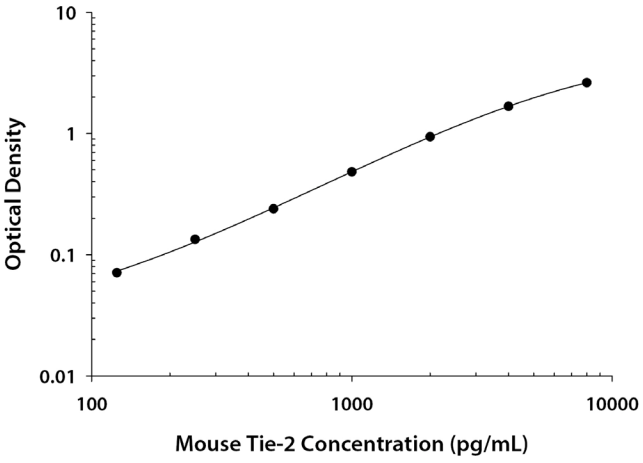
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 Tie-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	0.028 0.030	0.029	—
125	0.097 0.103	0.100	0.071
250	0.160 0.166	0.163	0.134
500	0.267 0.269	0.268	0.239
1000	0.485 0.538	0.512	0.483
2000	0.957 0.981	0.969	0.940
4000	1.622 1.785	1.704	1.675
8000	2.644 2.663	2.654	2.625

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-one 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	21	21	21
Mean (pg/mL)	395	1149	3470	434	1187	3183
Standard deviation	26.1	69.1	220	34.5	77.6	147
CV (%)	6.6	6.0	6.3	7.9	6.5	4.6

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	106	98-119%
Serum* (n=4)	103	93-116%
EDTA plasma* (n=5)	107	95-120%
Heparin plasma* (n=4)	102	87-113%

*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 various concentrations of mouse Tie-2 in each matrix were diluted with calibrator diluent and then assayed.

		Cell culture supernates (n=4)	Tissue homogenates* (n=3)	Serum* (n=5)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	92	92	104	96	98
	Range (%)	84-98	91-93	96-113	93-102	95-99
1:4	Average % of Expected	92	91	109	100	102
	Range (%)	86-101	90-92	105-113	95-106	92-120
1:8	Average % of Expected	98	90	106	99	100
	Range (%)	93-102	85-94	101-112	95-102	93-118
1:16	Average % of Expected	98	97	104	101	101
	Range (%)	87-104	93-104	94-115	96-109	96-108

*Samples were diluted prior to assay.

SENSITIVITY

Seventeen assays were evaluated and the minimum detectable dose (MDD) of mouse Tie-2 ranged from 6.3-40.2 pg/mL. The mean MDD was 18.9 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 mouse Tie-2 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for detectable levels of mouse Tie-2 in the assay. Serum and plasma samples are not matched.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Adult serum (n=12)	2431	2052-2786
Newborn serum (n=8)	5404	3702-7196
EDTA plasma (n=20)	1920	1476-2338
Heparin plasma (n=20)	2068	1667-2602

Cell Culture Supernates:
Mouse lungs were chopped into 1-2 mm pieces and seeded into approximately 50 mL of RPMI supplemented with 10% fetal bovine serum. The cell culture supernate was removed after seven days, tested for mouse Tie-2, and measured 399 pg/mL.

Mouse kidneys and livers were chopped into 1-2 mm pieces and separately seeded into approximately 100 mL of RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, and L-glutamine. The cell culture supernates were removed after 3 days, tested for mouse Tie-2, and measured 1836 pg/mL and 946 pg/mL, respectively.

Tissue Homogenates - Homogenates from mouse liver, spleen, lung, and heart tissue were assayed for mouse Tie-2 and measured 29 ng/mL, 13 ng/mL, 7.5 ng/mL, and 1.6 ng/mL, respectively.

SPECIFICITY

This assay recognizes natural and recombinant mouse Tie-2.

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 mouse Tie-2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

Ang-3
CT-1
E-Selectin
G-CSF
gp130
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-8
IL-9
IL-10
IL-10 R
IL-12 p70
IL-13
IL-17
IL-18
M-CSF
MIP-1α
MIP-1β
MIP-2
PlGF-2
RANK
RANTES
SCF
TARC
TNF-α
TNF RI
TNF RII
Tpo
TRANCE
VEGF
VEGF-D
VEGF R1
VEGF R2
VEGF R3

Recombinant human:

Ang-1
Ang-2
Ang-4
Tie-1

Recombinant zebrafish:

Tie-2

Recombinant human Tie-2 cross-reacts approximately 0.7% in this assay.

Rat serum and plasma samples were tested in this assay. No detectable levels were observed.

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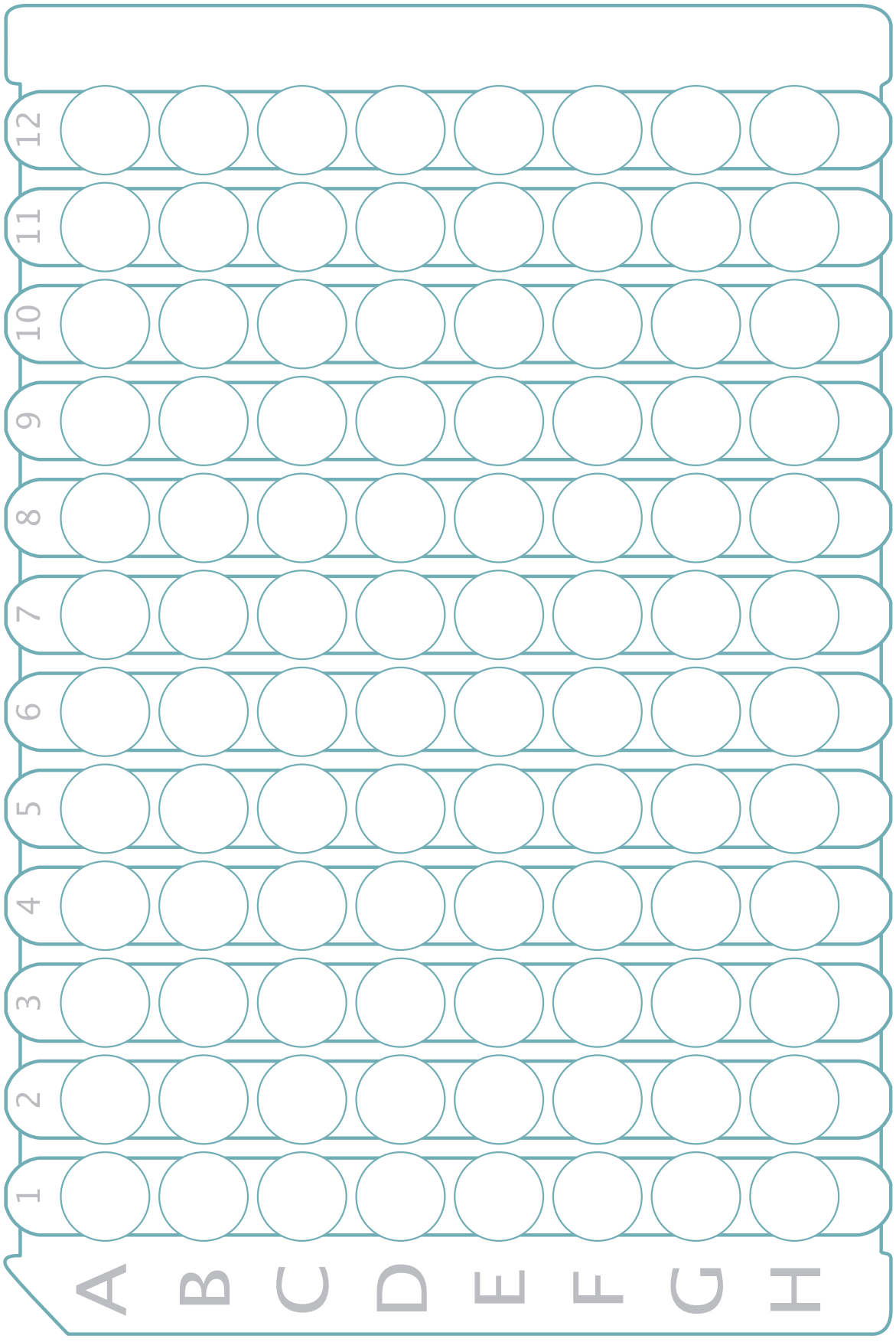
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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