Quantikine® ELISA

Mouse TIM-1/KIM-1/HAVCR Immunoassay

Catalog Number MKM100

For the quantitative determination of mouse T cell-Immunoglobulin-Mucin (TIM-1) concentrations in cell culture supernates, 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

T cell Immunoglobulin and Mucin domain 1 (TIM-1), also known as Kidney Injury Molecule 1 (KIM-1) and Hepatitis A Virus Cellular Receptor (HAVCR), is a member of the TIM family which is involved in the regulation of innate and adaptive immune responses (1, 2). Mouse TIM-1 is a type I transmembrane protein that contains an N-terminal immunoglobulin-like domain, a mucin domain with O- and N-linked glycosylations, a transmembrane segment, and a cytoplasmic signaling domain (3, 4). Multiple TIM-1 variants can be produced due to polymorphisms or alternate splicing resulting in deletions in the mucin domain (3). Some of these polymorphisms are associated with susceptibility to atopy, autoimmunity, and severe hepatitis A virus infection in humans (5). Within the extracellular domain, mouse TIM-1 shares 41% and 82% amino acid (aa) sequence identity with human and rat TIM-1, respectively.

Mouse TIM-1 is expressed on splenic B cells and is a marker for the identification of IL-10$^+$ regulatory B cells (6, 7). TIM-1 is also expressed on CD4$^+$ T cells, mast cells, invariant NKT (iNKT) cells, dendritic cells, kidney epithelium and a broad range of mucosal epithelium (4, 8-15). The expression of TIM-1 is upregulated on activated Th2 cells, after dendritic cell maturation, and on kidney tubular epithelial cells after injury (4, 9, 13, 14, 16, 17). Metalloproteinase-mediated cleavage of TIM-1 at the membrane-proximal region results in the release of a soluble form of TIM-1 which is detectable in the urine and in circulation (18, 19). Urinary TIM-1 is highly elevated in nephropathy and may be a useful biomarker for renal damage (16, 20-25).

TIM-1 has been reported to be a receptor for a number of ligands, including phosphatidylserine, leukocyte mono-immunoglobulin-like receptor 5 (LMIR5/CD300b), TIM-4, IgA, and the glycoproteins of a number of enveloped viruses (5, 15, 26-33). Its interaction with phosphatidylserine enables TIM-1 to mediate the phagocytosis of apoptotic cells (26-28). In TIM-1-bearing iNKT cells, interaction with apoptotic cells can also result in iNKT cell activation, proliferation, and cytokine production (11). Interactions between cell-surface or soluble TIM-1 with LMIR5 is proposed to induce LMIR5-mediated activation of myeloid cells including macrophages/monocytes, mast cells, neutrophils, and dendritic cells (29). These interactions contribute to tissue homeostasis and damage during kidney injury (29). Ligand-induced TIM-1 signaling co-stimulates T cell activation and enhances Th2 cytokine production (9, 31, 34). In humans, TIM-1 serves as a cellular entry receptor for various viruses, including hepatitis A virus, *Ebolavirus,* and *Marburgvirus* (15, 33).

The Quantikine® Mouse TIM-1/KIM-1/HAVCR Immunoassay is a 3.5 hour solid phase ELISA designed to measure mouse TIM-1 in cell culture supernates, serum, plasma, and urine. It contains NS0-expressed recombinant mouse TIM-1 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant factor accurately. Results obtained using natural mouse TIM-1 showed dose-response 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 TIM-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse TIM-1 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any TIM-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse TIM-1 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 TIM-1 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, 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.
- 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 TIM-1 Microplate	893809	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse TIM-1.	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.*
Mouse TIM-1 Conjugate	893810	12 mL of a monoclonal antibody specific for mouse TIM-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse TIM-1 Standard	893811	Recombinant mouse TIM-1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse TIM-1 Control	893812	Recombinant mouse TIM-1 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1-55	895066	11 mL of a buffered protein base with blue dye and preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:4 in this assay.*	
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.
- 100 mL and 500 mL graduated cylinders.
- 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 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. 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.*
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 precipitates that may appear after storage.

SAMPLE PREPARATION

Urine samples require at least a 15-fold dilution. A suggested 15-fold dilution is 10 µL of sample + 140 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

* See Reagent Preparation section.

REAGENT PREPARATION

The conjugate must be kept cold during use. Bring all other reagents to room temperature before use.

Mouse TIM-1 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

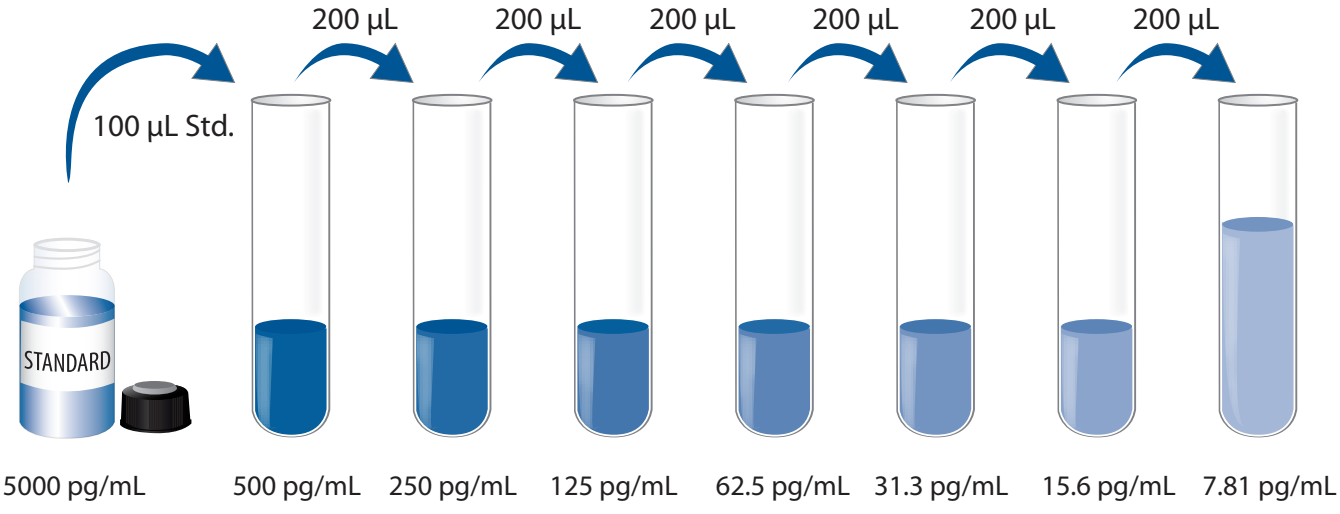
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-26 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-26 to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

Mouse TIM-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse TIM-1 Standard with Calibrator Diluent RD5-26 (diluted 1:4). This reconstitution produces a stock solution of 5000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-26 (diluted 1:4) into the 500 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 pg/mL standard serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

The conjugate must be kept cold during use. Bring all other 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, working standards, 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 RD1-55 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 the 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 **cold** Mouse TIM-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for **1 hour at 2-8 °C without shaking.**
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 Sample Preparation section.

CALCULATION OF RESULTS

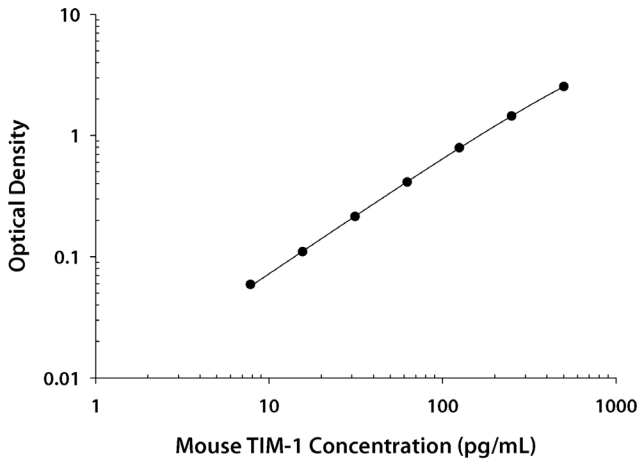
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 TIM-1 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	0.016 0.016	0.016	—
7.81	0.070 0.080	0.075	0.059
15.6	0.126 0.126	0.126	0.110
31.3	0.229 0.232	0.231	0.215
62.5	0.425 0.433	0.429	0.413
125	0.802 0.813	0.808	0.792
250	1.462 1.463	1.463	1.447
500	2.533 2.565	2.549	2.533

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)	24.2	61.8	207	23.2	60.0	190
Standard deviation	0.8	1.6	6.8	2.5	4.2	13.2
CV (%)	3.3	2.6	3.3	10.8	7.0	6.9

RECOVERY

The recovery of mouse TIM-1 spiked into various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	100	91-106%
Serum (n=4)	95	93-97%
EDTA plasma (n=4)	89	88-91%
Heparin plasma (n=4)	87	86-89%
Urine* (n=4)	102	94-115%

*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 TIM-1 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture samples * (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine* (n=4)
1:2	Average % of Expected	98	103	109	103	100
	Range (%)	97-101	101-104	106-115	101-107	96-109
1:4	Average % of Expected	99	102	111	106	100
	Range (%)	98-100	99-104	110-115	101-113	84-110
1:8	Average % of Expected	101	99	108	104	106
	Range (%)	99-104	96-102	101-116	98-113	105-107
1:16	Average % of Expected	103	96	106	107	105
	Range (%)	100-108	91-99	102-108	97-115	104-105

*Samples were diluted prior to assay.

SENSITIVITY

Fifty-four assays were evaluated and the minimum detectable dose (MDD) of mouse TIM-1 ranged from 0.22-2.18 pg/mL. The mean MDD was 0.71 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-derived recombinant mouse TIM-1 (Accession # Q3V033 amino acids Y22-T212; isoform 2) produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Urine - Samples were evaluated for the presence of mouse TIM-1 in this assay.

NSA Strain	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=20)	17.2	90	ND-42.5
EDTA plasma (n=20)	25.6	100	7.87-174
Heparin plasma (n=20)	18.0	95	ND-42.8
Urine (n=20)	2248	100	456-8048

ND=Non-detectable

Serum samples from two Balb/c strain mice were evaluated in this assay and measured 27.5 pg/mL and 26.9 pg/mL. Additionally, serum samples from two C57 strain mice were evaluated in this assay and measured 20.4 pg/mL and 14.4 pg/mL.

Urine samples from two Balb/c strain mice and one C57 strain mouse were evaluated in this assay and measured 143,000 pg/mL, 159,000 pg/mL, and 726 pg/mL respectively.

Cell Culture Supernates - Kidneys from mice were removed, rinsed in 1X PBS, and kept on ice in 1X PBS. Kidneys were then homogenized using a tissue homogenizer and seeded into media containing RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured for the times indicated. Aliquots of the cell culture supernates were removed and assayed for levels of mouse TIM-1.

Tissue Type	(pg/mL)
NSA mouse kidney (1 day)	1006
NSA mouse kidney (3 days)	756
Balb/c mouse kidney (2 days)	1679
C57 mouse kidney (2 days)	24

SPECIFICITY

This assay recognizes natural and recombinant mouse TIM-1.

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 mouse TIM-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

Cystatin C
Lipocalin-2/NGAL
MMP-2
MMP-3
MMP-7
MMP-8
MMP-9
MMP-12
TIM-2
TIM-3
TIM-4
TIM-5
TIM-6
TIM-7

Recombinant rat:

Lipocalin-2/NGAL
TIM-1/KIM-1/HAVCR

Recombinant human:

TIM-1/KIM-1/HAVCR
TIM-3
TIM-4

The antibody components of this kit were raised against the short splice form of mouse TIM-1 (isoform 2) which has a 23 aa deletion following Pro182. This immunoassay detects both the short and long forms of mouse TIM-1.

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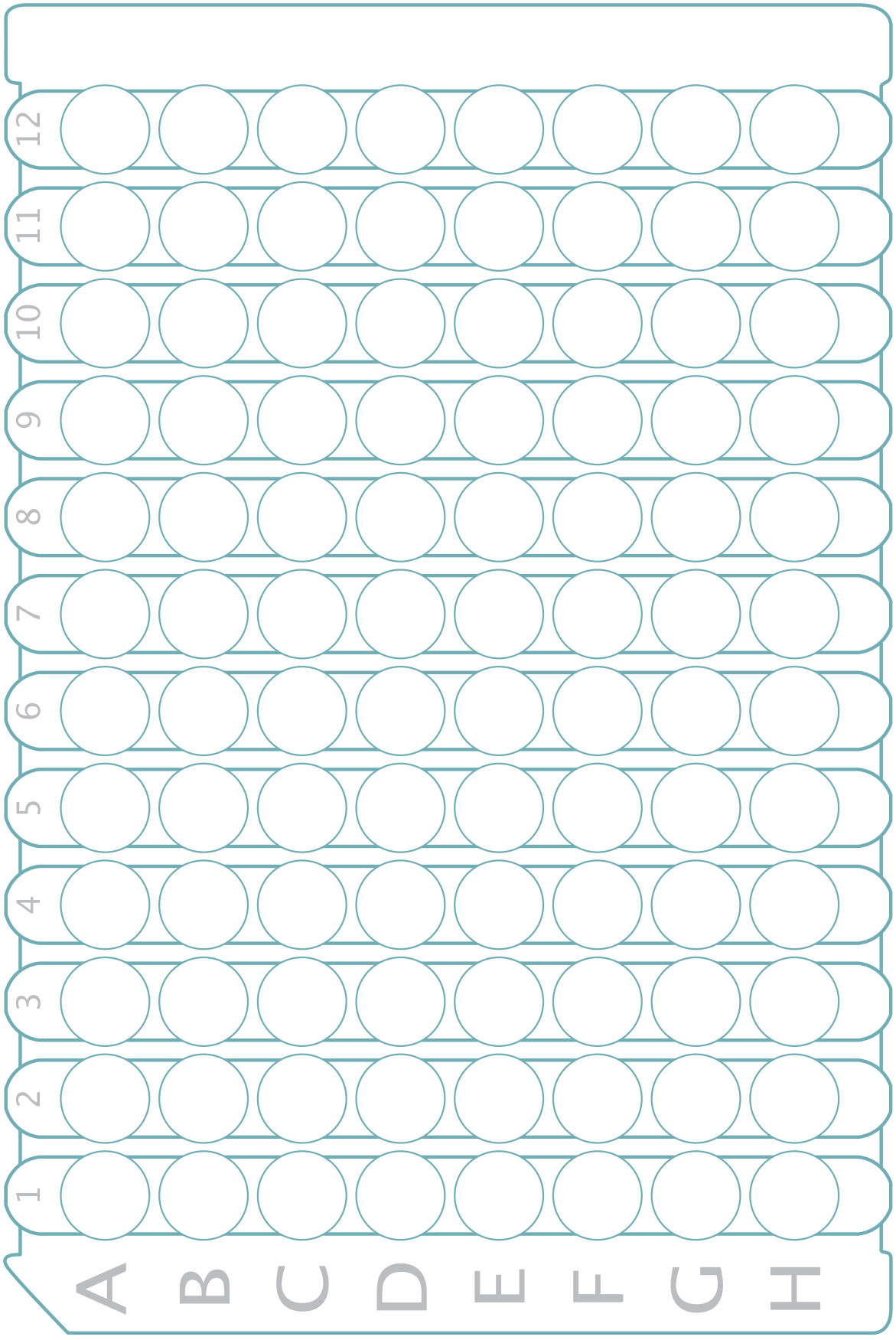
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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