Quantikine® ELISA

Rat TIM-1/KIM-1/HAVCR Immunoassay

Catalog Number RKM100

For the quantitative determination of rat T cell Immunoglobulin Mucin (TIM-1) concentrations in cell culture supernates, tissue homogenates, 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 1 (HAVcr1), is a member of the TIM family which is involved in the regulation of innate and adaptive immune responses (1, 2). 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 alternative 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, rat TIM-1 shares 82% and 41% amino acid (aa) sequence identity with mouse and human TIM-1, respectively.

In vivo, 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-1, 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 costimulates 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® Rat TIM-1/KIM-1/HAVCR Immunoassay is a 4.5 hour solid-phase ELISA designed to measure rat TIM-1 in cell culture supernates, tissue homogenates, serum, plasma, and urine. It contains NS0-expressed recombinant rat TIM-1 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural rat TIM-1 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 rat TIM-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat 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 polyclonal antibody specific for rat 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, 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.
- 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.

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.

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
Rat TIM-1 Microplate	893428	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for rat TIM-1.	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.*
Rat TIM-1 Conjugate	893429	12 mL of a polyclonal antibody specific for rat TIM-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Rat TIM-1 Standard	893430	Recombinant rat TIM-1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Rat TIM-1 Control	893431	Recombinant rat 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 RD1W	895038	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-3	895436	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.

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 - Prior to assay, tissues must be lysed according to the directions in the Sample Values section.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 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 EDTA or heparin as an anticoagulant. Centrifuge for 20 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 - 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

Serum, plasma, and urine samples require a 2-fold dilution. A suggested 2-fold dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD5-3.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

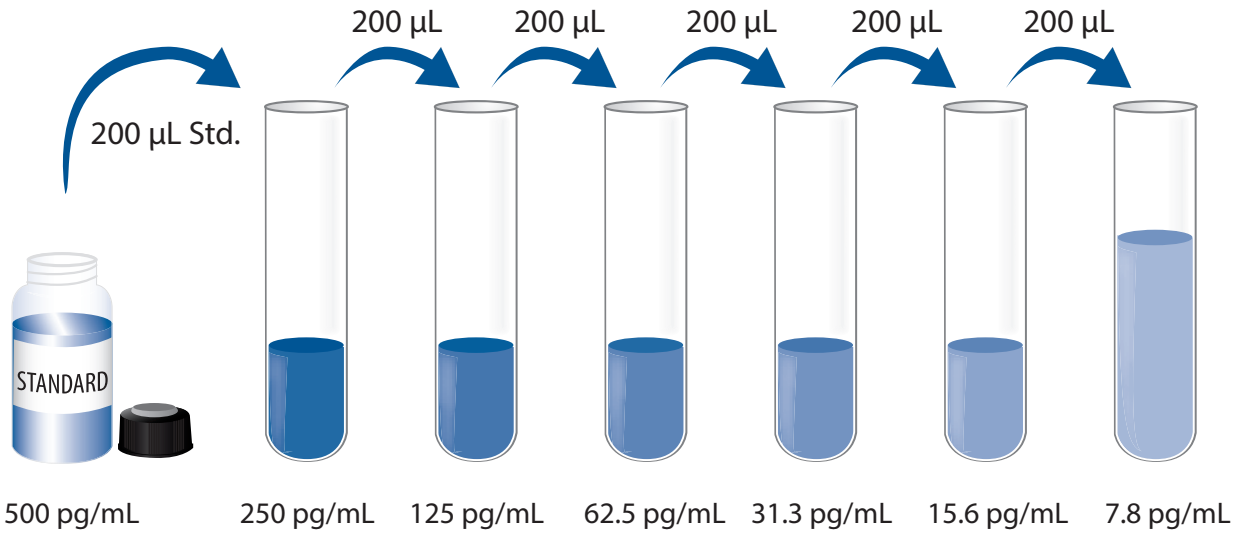
Rat TIM-1 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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.

Rat TIM-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Rat TIM-1 Standard with Calibrator Diluent RD5-3. Do not substitute other diluents. This reconstitution produces a stock solution of 500 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 RD5-3 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Rat TIM-1 Standard (500 pg/mL) serves as the high standard. Calibrator Diluent RD5-3 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 RD1W 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.
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 Rat TIM-1 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 Sample Preparation section.

CALCULATION OF RESULTS

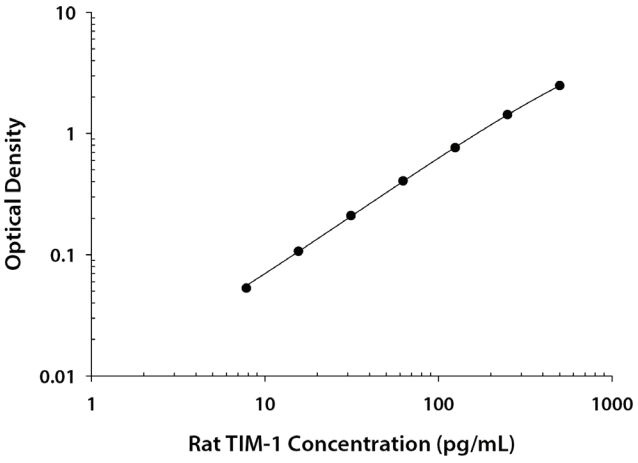
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 rat 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	0.060 0.062	0.061	—
7.8	0.113 0.114	0.114	0.053
15.6	0.167 0.169	0.168	0.107
31.3	0.270 0.271	0.271	0.210
62.5	0.465 0.468	0.467	0.406
125	0.819 0.830	0.825	0.764
250	1.486 1.496	1.491	1.430
500	2.510 2.573	2.542	2.481

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 forty 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	40	40	40
Mean (pg/mL)	21.8	54.4	161	26.2	60.0	169
Standard deviation	0.80	2.45	5.22	2.32	3.79	7.51
CV (%)	3.7	4.5	3.2	8.9	6.3	4.4

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates (n=5)	96	93-99%
Serum* (n=4)	102	82-112%
EDTA plasma* (n=4)	99	84-107%
Heparin plasma* (n=4)	102	95-114%
Urine* (n=4)	103	87-117%

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

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of rat TIM-1 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=5)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine* (n=4)
1:2	Average % of Expected	99	99	102	101	97
	Range (%)	97-103	96-100	100-103	98-107	93-103
1:4	Average % of Expected	102	103	105	102	99
	Range (%)	97-108	99-107	103-106	98-107	94-104
1:8	Average % of Expected	105	107	107	103	102
	Range (%)	99-114	98-119	104-110	97-111	95-111
1:16	Average % of Expected	108	106	106	101	103
	Range (%)	102-117	97-112	101-110	95-111	94-110

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

SENSITIVITY

Ninety-one assays were evaluated and the minimum detectable dose (MDD) of rat TIM-1 ranged from 0.30-2.74 pg/mL. The mean MDD was 0.96 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 rat TIM-1 produced at R&D Systems®.

SAMPLE VALUES

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

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=17)	307	233-471	59.7
EDTA plasma (n=20)	265	160-400	59.0
Heparin plasma (n=20)	247	127-402	66.0
Urine (n=20)	666	325-1993	509

Cell Culture Supernates - Rat spleen cells were cultured for 1 day in RPMI 1640 supplemented with 10% fetal bovine serum. The cells were cultured unstimulated or stimulated with 1.0 µg/mL of lipopolysaccharide (LPS), 10 µg/mL of Concanavalin A (ConA), or 10 µg/mL of PHA and 10 ng/mL of PMA. Aliquots of the cell culture supernates were removed and assayed for levels of rat TIM-1.

	(pg/mL)
Unstimulated	21.9
1.0 µg/mL LPS	26.9
10 µg/mL ConA	30.0
10 µg/mL PHA & 10 ng/mL PMA	37.8

Tissue Homogenates - One rat kidney was cut into pieces (1-2 mm) and kept on ice. The tissue was rinsed once with PBS and the wash was discarded. 4.0 mL of PBS was added, the tissue was homogenized, and centrifuged to remove debris. An aliquot of the tissue homogenate supernate was removed, assayed for rat TIM-1, and measured 55.9 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant rat 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 rat TIM-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant rat:

Cystatin C
IFN-γ
IL-4
IL-5
IL-6
IL-13
MMP-8
MMP-9
NGAL
TNF-α

Recombinant mouse:

MMP-2
MMP-3
MMP-7
MMP-8
MMP-9
MMP-12
TIM-1
TIM-2
TIM-3
TIM-4
TIM-5
TIM-6
TIM-7

Recombinant human:

TIM
TIM-1
TIM-3
TIM-4

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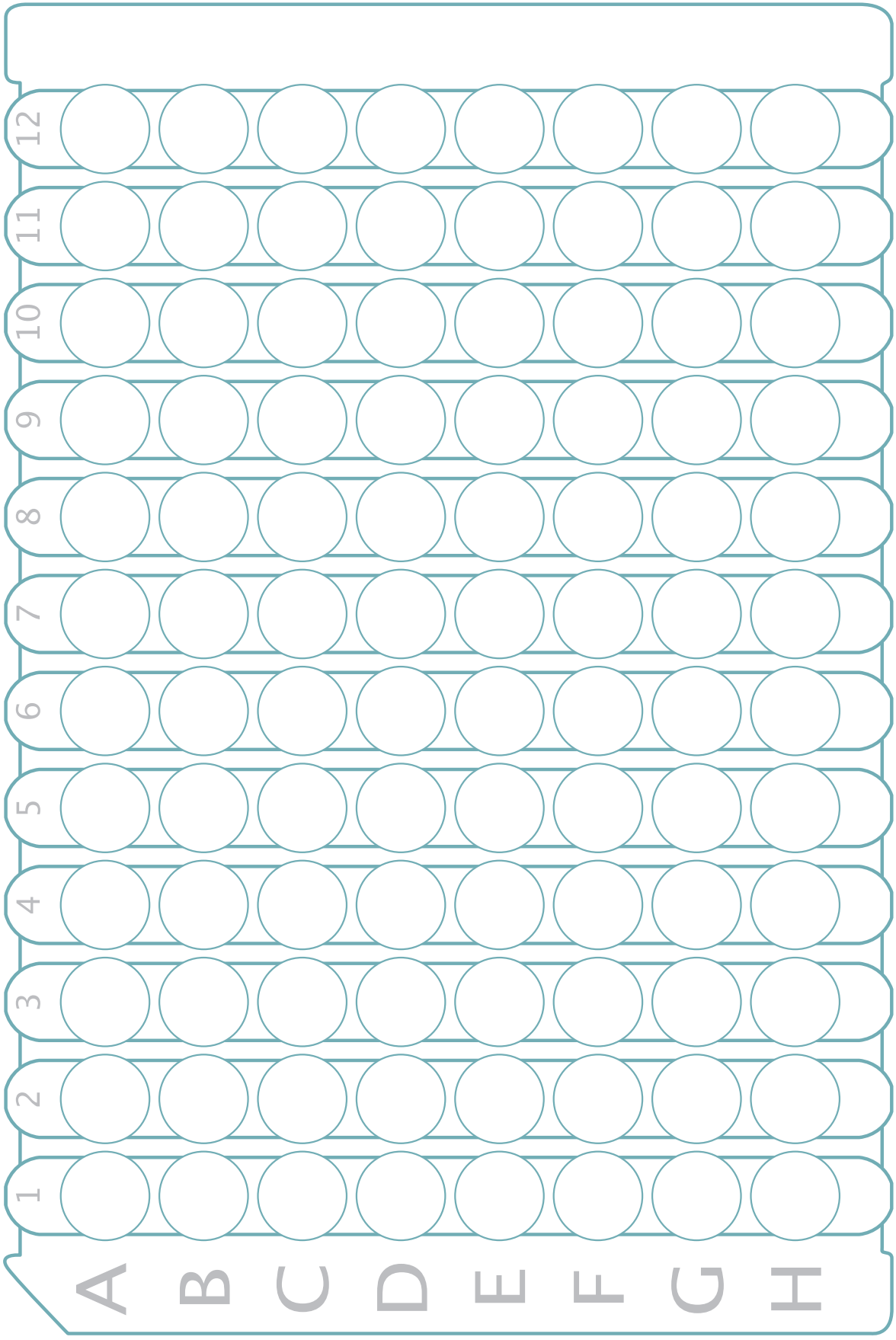
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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