Quantikine® ELISA

Rat TIMP-1 Immunoassay

Catalog Number RTM100
SRTM100
PRTM100

For the quantitative determination of rat Tissue Inhibitor of Metalloproteinases 1 (TIMP-1) concentrations in cell culture supernates, serum, and plasma.

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

Matrix metalloproteinases (MMPs), also called matrixins, constitute a family of zinc and calcium dependent endopeptidases that function in the breakdown of extracellular matrix and in the processing of a variety of biological molecules. They play an important role in many normal physiological processes such as embryonic development, morphogenesis, reproduction and tissue remodeling (1). They also participate in many pathological processes such as arthritis, cancer and cardiovascular disease (2). While the amounts of newly synthesized MMPs are regulated mainly at the levels of transcription, the proteolytic activities of existing MMPs are controlled through both the activation of proenzymes or zymogens and the inhibition of active enzymes by endogenous inhibitors such as α_2-macroglobulins and tissue inhibitors of metalloproteinases (TIMPs).

The mammalian TIMP family includes four members that share structural similarity (3). All TIMP proteins have 12 conserved cysteine residues that form six intrachain disulfide bonds, resulting in an extremely stable protein with six loops. The TIMP protein has two structurally and functionally distinct domains: the N-terminal domain consisting of loops 1-3 that are responsible for tight, but non-covalent binding to the active MMPs in a 1:1 stoichiometry; and the C-terminal domain consisting of loops 4-6 that enhances the enzyme-inhibitor interactions. In the case of TIMP-1, the C-terminal domain has also been shown to bind the hemopexin-like domain of pro-MMP-9. TIMP-1 stimulates erythropoiesis, inhibits angiogenesis and is an anti-apoptotic agent for B cells. These TIMP-1 functions may be independent of MMP inhibition (4-6).

Rat TIMP-1 is a 28-35 kDa secreted glycoprotein (6, 7). The protein is synthesized as a 217 amino acid (aa) precursor that contains a 23 aa signal peptide and a 194 aa mature form (8). Rat TIMP-1 shares 85%, 70%, 68%, and 66% aa sequence identity with its mouse, human, porcine, and canine counterparts, respectively (9-13). Among the three known rat TIMPs, TIMP-1 shares 40% and 35% aa identity with TIMP-2 and TIMP-3, respectively (14, 15). TIMP-1 is widely expressed in many cells such as fibroblasts, osteoblasts, endothelial cells, granulosa cells, dendritic cells, vascular smooth muscle cells, adipocytes, and monocytes (6, 16-19).

The Quantikine Rat TIMP-1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure rat TIMP-1 levels in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant rat TIMP-1 and antibodies raised against the recombinant protein. Natural rat TIMP-1 showed dose-response curves that were parallel to the standard curves obtained using the Quantikine kit standards, indicating that this kit can be used to determine relative levels of natural rat TIMP-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat TIMP-1 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any rat TIMP-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for rat TIMP-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 rat TIMP-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 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.
- 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 #	CATALOG # RTM100	CATALOG # SRTM100	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Rat TIMP-1 Microplate	892345	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for rat TIMP-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 TIMP-1 Conjugate	892346	1 vial	6 vials	12 mL/vial of a polyclonal antibody against rat TIMP-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Rat TIMP-1 Standard	892347	1 vial	6 vials	12 ng/vial of recombinant rat TIMP-1 in a buffered protein base with preservatives; lyophilized.	
Rat TIMP-1 Control	892348	1 vial	6 vials	Recombinant rat TIMP-1 in a buffered protein base with preservatives; lyophilized. The concentration range of rat TIMP-1 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 RD1-21	895215	1 vial	3 vials	12 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-17	895512	2 vials	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

RTM100 contains sufficient materials to run ELISAs on one 96 well plate.
SRTM100 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PRTM100). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

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.
- 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 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 and 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 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: *Heparin and citrate plasma have 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 20-fold dilution prior to assay. A suggested 20-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent RD5-17.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

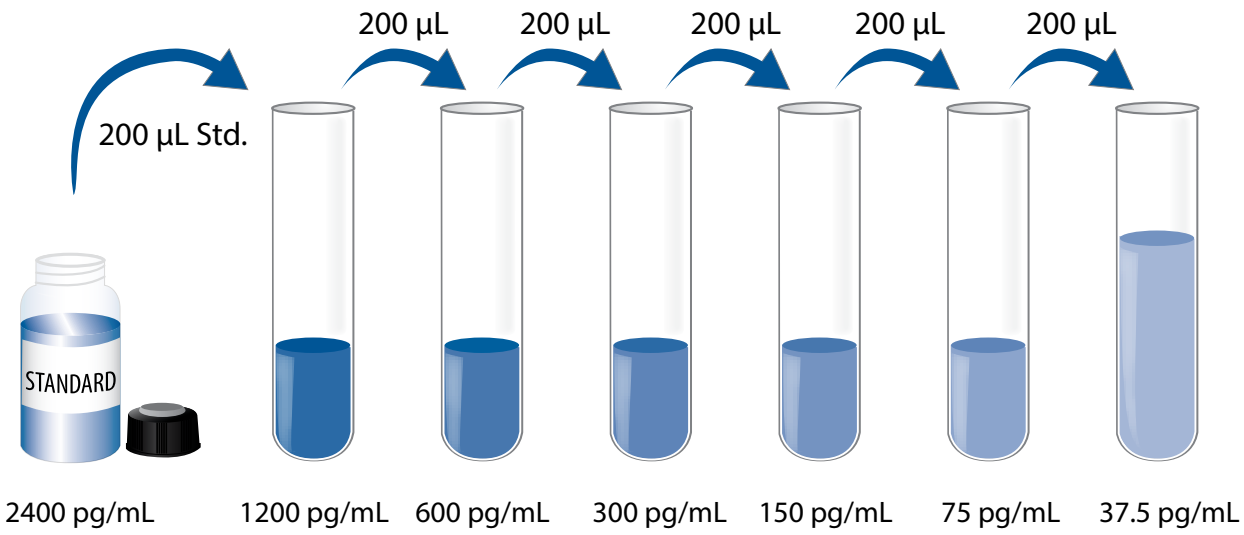
Rat TIMP-1 Control - Reconstitute the Control with 1.0 mL deionized or distilled water. Assay the Control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 20 mL 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.

Rat TIMP-1 Standard - Reconstitute the Rat TIMP-1 Standard with 5.0 mL of Calibrator Diluent RD5-17. Do not substitute other diluents. This reconstitution produces a stock solution of 2400 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 thoroughly before the next transfer. The undiluted Rat TIMP-1 Standard (2400 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 samples, standards, and control 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 RD1-21 to each well.
4. Add 50 µL of Standard, Control, or sample* per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. 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 rat TIMP-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
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. **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.

*Serum and plasma samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

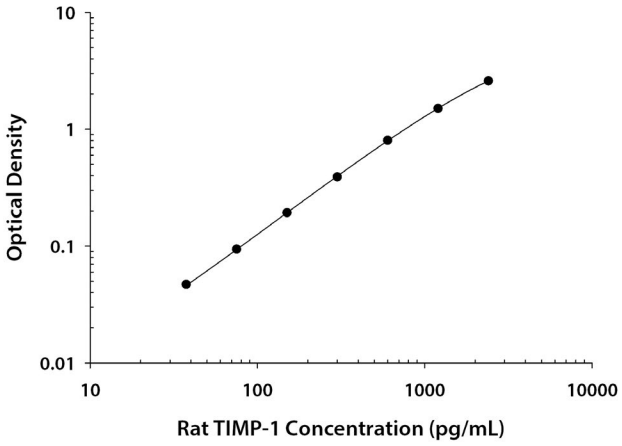
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 TIMP-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	0.037 0.040	0.039	—
37.5	0.083 0.088	0.086	0.047
75	0.130 0.135	0.133	0.094
150	0.231 0.233	0.232	0.193
300	0.423 0.437	0.430	0.391
600	0.837 0.846	0.842	0.803
1200	1.520 1.568	1.544	1.505
2400	2.604 2.657	2.631	2.592

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)	122	443	1307	132	445	1277
Standard deviation	4.5	14	34	9.6	27	85
CV (%)	3.7	3.2	2.6	7.3	6.1	6.7

RECOVERY

The recovery of rat TIMP-1 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=5)	106	93-119%
Serum* (n=7)	98	84-120%
EDTA plasma* (n=7)	99	82-115%

*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 various concentrations of rat TIMP-1 in each matrix were diluted with Calibrator Diluent and assayed.

		Cell culture supernates (n=4)	Serum* (n=5)	EDTA plasma* (n=6)
1:2	Average % of Expected	96	99	98
	Range (%)	89-102	93-106	95-102
1:4	Average % of Expected	100	98	96
	Range (%)	85-110	90-108	92-98
1:8	Average % of Expected	94	102	93
	Range (%)	84-98	91-110	87-98
1:16	Average % of Expected	92	101	92
	Range (%)	85-96	86-113	83-102

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

SENSITIVITY

Six assays were evaluated and the minimum detectable dose (MDD) of rat TIMP-1 ranged from 2.5-4.5 pg/mL. The mean MDD was 3.5 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 rat TIMP-1 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for detectable levels of rat TIMP-1 in the assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=20)	17,000	9800-25,900	5200
EDTA plasma (n=20)	13,000	7400-41,400	7200

Cell Culture Supernates:
Rat lung was cultured in DMEM supplemented with 10% fetal bovine serum for 3-5 days. An aliquot of the cell culture supernate was removed, assayed for rat TIMP-1, and measured 4900 pg/mL.

Rat splenocytes were cultured in DMEM supplemented with 10% fetal bovine serum for 3-5 days. An aliquot of the cell culture supernate was removed, assayed for rat TIMP-1, and measured 2200 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant rat TIMP-1.

The factors listed below were prepared at 50 ng/mL or 500 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a recombinant rat TIMP-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

MMP-3
MMP-9 (active)
MMP-9 (pro)

Recombinant human:

MMP-1
MMP-2
MMP-3
MMP-7
MMP-8
MMP-9
MMP-10
MMP-12
MMP-13
TIMP-1
TIMP-2
TIMP-3
TIMP-4

Recombinant mouse TIMP-1 cross-reacts approximately 0.015% in this assay.

A constant amount of recombinant rat (rr) TIMP-1 (1 µg/mL) was mixed with the pro- or active form of recombinant mouse MMP-9 at a molar ratio of 1:10 and incubated at 2-8 °C overnight. The complex was diluted 1000-fold, and rrTIMP-1 was measured in the assay. The measured rrTIMP-1 concentration in the pro-MMP-9 complex was not significantly changed from the free rrTIMP-1. However, when complexed with the active MMP-9 at a 1:10 molar ratio, 77% of the expected TIMP-1 value was observed.

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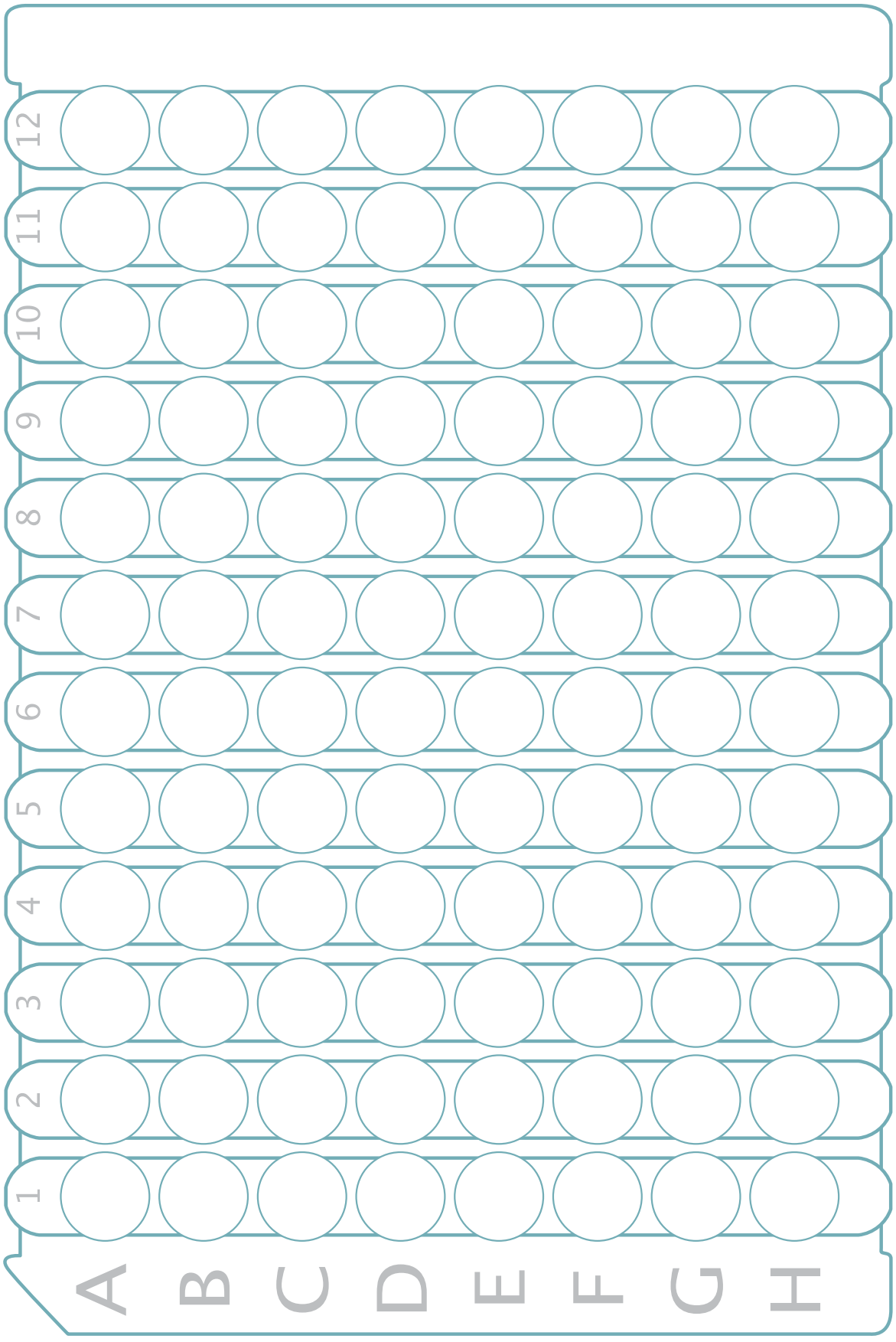
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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