Quantikine™ ELISA

Human TIMP-1 Immunoassay

Catalog Number DTM100
STM100
PDTM100

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

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) are zinc-dependent endopeptidases that catalyze degradation of extracellular matrix proteins, thereby controlling such processes as development, tissue remodeling, wound healing and tumor metastasis (1-3). The activity of MMPs is controlled by regulation of expression and secretion, by proteolytic activation of pro-enzymes and by the Tissue Inhibitors of Metalloproteinases (TIMPs) (4, 5). TIMPs form 1:1, non-covalent complexes with MMPs, blocking access of substrates to the MMP catalytic site. TIMPs are highly specific for MMPs in general but not for any particular MMP. Functional specificity is conferred by other characteristics. TIMP-1 is an inducible protein and TIMP-2 is a constitutive protein and both are soluble and widely distributed. TIMP-3 is restricted to the extracellular matrix and TIMP-4 is largely restricted to cardiac tissue. For reviews on MMPs and TIMPs, see references 1-5.

TIMP-1 is a 184 amino acid residue glycosylated protein, though glycosylation is not necessary for activity (6). It has 12 cysteines (conserved among all TIMPs) that form disulfide bonds in a pattern that gives distinct N- and C-terminal domains (7). The N-terminal domain contains sites that bind to the MMP substrate-binding site (8). Binding of TIMP-1 does not leave a peptide bond in position for proteolysis and is not cleaved (5). The TIMP/MMP complex can dissociate to yield enzyme and active TIMP-1 (9). The C-terminal domain binds to an external site on MMPs, increasing overall affinity (5). TIMP-1 binds with high affinity to the inactive pro-MMP-9, forming a complex in which TIMP-1 retains its ability to inhibit the activity of another active MMP via its N-terminal domain (10).

TIMP-1 is widely synthesized by many cells and tissues (4). Transcription of the TIMP-1 gene is induced by pro-inflammatory cytokines (IL-1, IL-6, OSM, LIF and TNF-α), TGF-β1 and phorbol esters (4, 11). Many physiological functions of TIMP-1 are closely tied to the functions of MMPs, and an improper balance of MMP and TIMP production correlates with pathological conditions such as arthritis, tumor growth and metastasis (4). On the other hand, TIMP-1 was independently discovered as an erythroid potentiating activity (12, 13), an activity that appears to be functionally distinct from MMP inhibitory activity (14). TIMP-1 binds to certain cell lines and is translocated to the nucleus (15). It inhibits apoptosis in B cells (16), further suggesting that it independently functions in multiple ways to support survival and growth of cells in contrast to its function of inhibition of MMPs.

The Quantikine™ Human TIMP-1 Immunoassay is a 3.5 hour solid phase ELISA designed to measure human TIMP-1 concentrations in cell culture supernates, serum, plasma, and saliva. It contains NS0-expressed recombinant human TIMP-1 and antibodies raised against the recombinant factor. It has been shown to quantitate recombinant human TIMP-1 accurately. Results obtained using natural TIMP-1 showed linear curves that were parallel to the standard curve obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural human TIMP-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human TIMP-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TIMP-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human 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 and color develops in proportion to the amount of TIMP-1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

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.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DTM100	CATALOG # STM100	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human TIMP-1 Microplate	890054	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human 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.*
Human TIMP-1 Conjugate	890055	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human TIMP-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human TIMP-1 Standard	890056	1 vial	6 vials	Recombinant human TIMP-1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1X	895121	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives. *May contain crystals. Mix well before and during use.*	
Calibrator Diluent RD5P	895151	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
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	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DTM100 contains sufficient materials to run an ELISA on one 96 well plate.
STM100 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDTM100). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human TIMP-1 Microplate	890054	50 plates
Human TIMP-1 Conjugate	890055	50 vials
Human TIMP-1 Standard	890056	25 vials
Assay Diluent RD1X	895121	50 vials
Calibrator Diluent RD5P	895151	50 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets
Package Inserts	750038	2 booklets

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 shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Collection device for saliva samples that has no enzyme binding or filtering capabilities such as Salivette® or equivalent
- Test tubes for dilution of standards and samples
- Human TIMP-1 Controls (optional; R&D Systems®, Catalog # QC173)

PRECAUTIONS

High levels of TIMP-1 are found in saliva. Take necessary precautions (e.g. mask and gloves) to prevent contamination of the kit reagents while running this assay.

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

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 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 15 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.*
Grossly lipemic samples should not be used in this assay.

Saliva - Collect saliva using a collection device such as a Salivette or equivalent. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Saliva collector cannot have any enzyme binding or filtering capabilities.*

SAMPLE PREPARATION

All samples require a 100-fold dilution in Calibrator Diluent RD5P (diluted 1:5). A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5P (diluted 1:5).

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High levels of TIMP-1 are found in saliva. Take necessary precautions (e.g. mask and gloves) to protect kit reagents.*

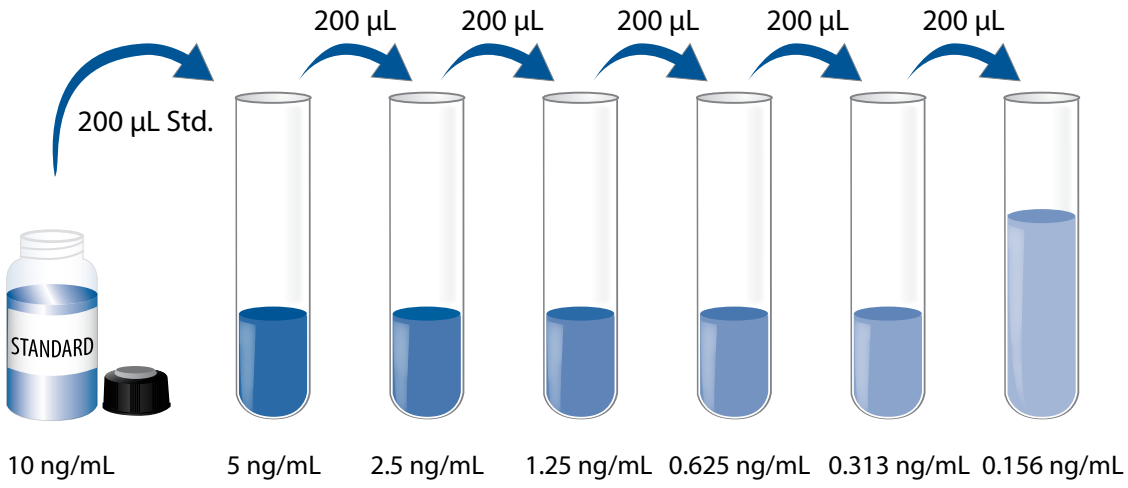
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Human TIMP-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human TIMP-1 Standard with deionized or distilled water. This reconstitution produces a stock solution of 10 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5P (diluted 1:5) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard stock serves as the high standard (10 ng/mL). Calibrator Diluent RD5P (diluted 1:5) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

Note: *High levels of TIMP-1 are found in saliva. Take necessary precautions (e.g. mask and gloves) to protect kit reagents.*

1. Prepare all reagents, working standards, 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 100 µL of Assay Diluent RD1X to each well. *Assay Diluent RD1X may contain a crystalline precipitate. Mix well before and during use.*
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 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 two times for a total of three 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 200 µL of Human TIMP-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, 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 require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

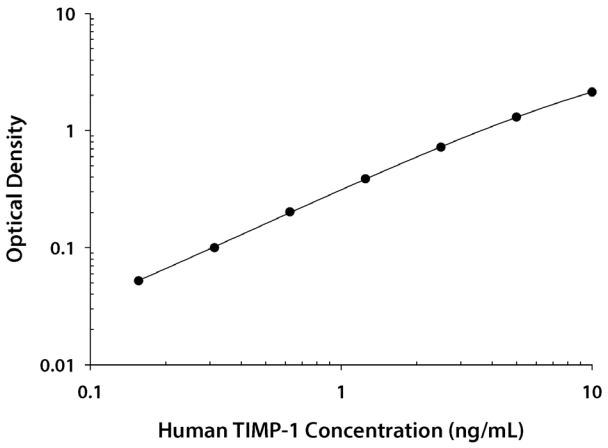
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human 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.

Since 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.



(ng/mL)	O.D.	Average	Corrected
0	0.033 0.034	0.034	—
0.156	0.086 0.087	0.086	0.052
0.313	0.133 0.135	0.134	0.100
0.625	0.235 0.237	0.236	0.202
1.25	0.419 0.423	0.421	0.387
2.5	0.740 0.774	0.757	0.723
5	1.324 1.347	1.336	1.302
10	2.142 2.190	2.166	2.132

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 (ng/mL)	0.48	1.27	6.95	0.51	1.28	6.90
Standard deviation	0.02	0.05	0.35	0.02	0.05	0.34
CV (%)	4.2	3.9	5.0	3.9	3.9	4.9

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	102	94-113%
Serum* (n=5)	98	87-102%
Heparin plasma* (n=5)	99	89-108%
EDTA plasma* (n=5)	99	93-108%
Saliva* (n = 4)	105	89-121%

*Samples were initially 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 human TIMP-1 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=10)	Serum* (n=9)	EDTA plasma* (n=9)	Heparin plasma* (n=9)	Saliva* (n=8)
1:2	Average % of Expected	100	103	102	104	101
	Range (%)	97-105	100-110	98-108	100-108	94-105
1:4	Average % of Expected	100	103	103	103	100
	Range (%)	96-106	98-111	96-110	95-110	96-109
1:8	Average % of Expected	103	101	100	103	100
	Range (%)	89-110	93-110	92-110	94-111	95-113
1:16	Average % of Expected	104	100	100	100	101
	Range (%)	91-112	93-108	93-108	85-111	87-115

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

SENSITIVITY

The minimum detectable dose (MDD) of human TIMP-1 is typically less than 0.08 ng/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 human TIMP-1 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Saliva - Samples from apparently healthy volunteers were evaluated for the presence of human TIMP-1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=60)	190	87-524	72.1
Heparin plasma (n=60)	84	39-279	33.1
EDTA plasma (n=60)	98	44-304	35.4
Saliva (n=4)	121	46-208	82.3

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the culture supernate were removed on days 1 and 5 and assayed for levels of natural human TIMP-1.

Condition	Day 1 (ng/mL)	Day 5 (ng/mL)
Unstimulated	76	163
Stimulated	201	274

SPECIFICITY

This assay recognizes natural and recombinant human (rh) TIMP-1.

Related factors were prepared at 200 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the related factors at 200 ng/mL in a mid-range recombinant human TIMP-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

MMP-1
MMP-2
MMP-3
TIMP-2
TIMP-3
TIMP-4

No cross-reactivity was observed with rhMMP-9, but interference was observed at concentrations ≥ 100 ng/mL.

rhMMP-9 Concentration (ng/mL)	Observed TIMP-1 Value (ng/mL)
200	227.6
100	249.8
50	263.4
25	269.8
12.5	287.0
0	276.9

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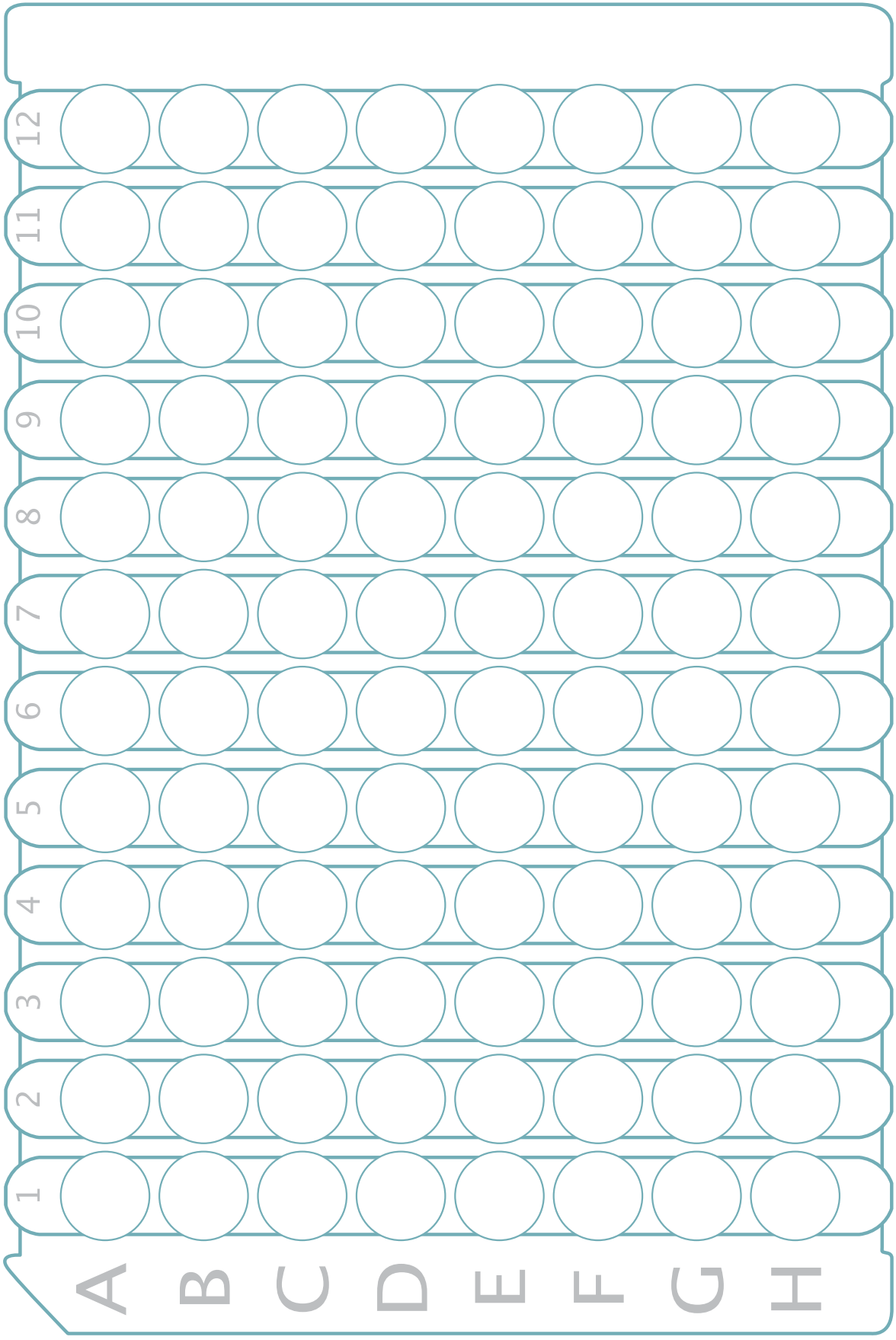
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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