Quantikine® ELISA

Mouse MSP/MST1 Immunoassay

Catalog Number MMSP0

For the quantitative determination of mouse Macrophage Stimulating Protein (MSP) concentrations in cell culture supernates, tissue lysates, 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

Macrophage stimulating protein (MSP), also known as HGF-like protein, and scatter factor-2, is a member of the HGF family of growth factors (1, 2). MSP contains a PAN/APPLE-like domain, four kringle domains, and a peptidase S1 domain which lacks enzymatic activity (3). Mouse MSP shares 79% and 93% amino acid (aa) sequence identity with human and rat MSP, respectively, and 42% aa sequence identity with mouse HGF. MSP is secreted by hepatocytes into the circulation as an inactive pro-protein (4, 5). Circulating pro-MSP is proteolytically cleaved in response to tissue injury to yield biologically active disulfide linked heterodimers consisting of a 45-62 kDa alpha chain and a 25-35 kDa beta chain (6, 7). The substitution of cysteine 672 (in the beta chain) with alanine significantly increases the bioactivity of recombinant MSP, apparently by limiting incorrect disulfide bond formation between the alpha and beta chains (8). Pro-MSP can be activated by many proteases including HGF Activator, Matriptase, Hepsin, Coagulation Factors XIa and XIIa, Kallikrein 1, Kallikrein 1B3/NGF-gamma, Kallikrein 3/PSA, and human airway trypsin-like protease/TMPRSS11D (5, 9-13). MSP levels are elevated in the serum during acute renal failure (14) as well as in the bronchiolar lavage fluid of sarcoidosis and bronchiectasis patients (15, 16).

Heterodimeric MSP, as well as the isolated beta chain, binds to the receptor tyrosine kinase MSP R/Ron with high-affinity, although only heterodimeric MSP can induce receptor signaling (17-20). Mature mouse MSP R consists of a 281 aa extracellular alpha chain and a 1068 aa transmembrane beta chain which are attached with a disulfide bond linkage. A secreted soluble isoform of MSP R binds heterodimeric MSP and inhibits MSP R-dependent signaling pathways (21). MSP R is expressed on macrophages and epithelial cells in the skin, lung, digestive tract, and the female reproductive tract (17, 22). MSP R is upregulated in many carcinomas, and its activation by MSP contributes to tumor metastasis (2). MSP R associates with a variety of transmembrane proteins including integrins, cadherins and other cytokine receptors. These complexes can be transactivated by ligands of either receptor component. Their activation induces signaling crosstalk between MSP R and the associated transmembrane receptors (23-26).

MSP exerts both positive and negative effects on macrophage mediated inflammation (1, 2). It enhances macrophage migration, respiratory burst, and phagocytosis of complement-opsonized particles (5, 6, 27). In contrast, it inhibits LPS- or IFN-induced inflammatory mediator production by macrophages and rheumatoid arthritis synovial fibroblasts (11, 28, 29). MSP promotes the proliferation of keratinocytes, renal tubule epithelial cells, and renal mesangial cells (14, 30, 31), and enhances osteoclast mediated bone resorption (32). MSP inhibits the differentiation and proliferation of early hematopoietic myeloid progenitors (33) but enhances the differentiation of megakaryocytes and erythroid cells (34, 35). MSP functions as a neurotrophic factor, promoting neuronal survival as well as neurite extension and branching of sensory and sympathetic neurons (36, 37).

The Quantikine Mouse MSP/MST1 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse MSP levels in cell culture supernates, tissue lysates, serum, plasma, and urine. It contains CHO cell-expressed recombinant mouse MSP and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse MSP accurately. Results obtained using natural mouse MSP showed dose-response curves that were parallel to the standard curves obtained using the recombinant kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse MSP.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse MSP has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse MSP present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse MSP 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 mouse MSP 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.
- 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 MSP Microplate	894561	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse MSP.	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 MSP Conjugate	894562	12 mL of a polyclonal antibody specific for mouse MSP conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse MSP Standard	894563	100 ng of recombinant mouse MSP in a buffered protein base with preservatives; lyophilized.	
Mouse MSP Control	894564	Recombinant mouse MSP in a buffered protein base with preservatives; lyophilized. The concentration range of mouse MSP after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.	
Calibrator Diluent RD5-24	895325	21 mL of a concentrated 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.
- **Polypropylene** test tubes for dilution of standards and samples.

If using tissue lysate samples, the following are also required:

- Cell Lysis Buffer 1 (R&D Systems, Catalog # 890713).
- PBS

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

Tissue Lysates - 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 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.*

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

Cell culture supernate, tissue lysate, and urine samples require a 2-fold dilution. A suggested 2-fold dilution is 125 µL of sample + 125 µL of Calibrator Diluent RD5-24 (1:10).

Serum and plasma samples require a 400-fold dilution. A suggested 400-fold dilution can be achieved by adding 10 µL of sample to 490 µL of Calibrator Diluent RD5-24 (1:10). Complete the 400-fold dilution by adding 75 µL of the diluted sample to 525 µL Calibrator Diluent RD5-24 (1:10).

REAGENT PREPARATION

Bring all reagents to room temperature before use.

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

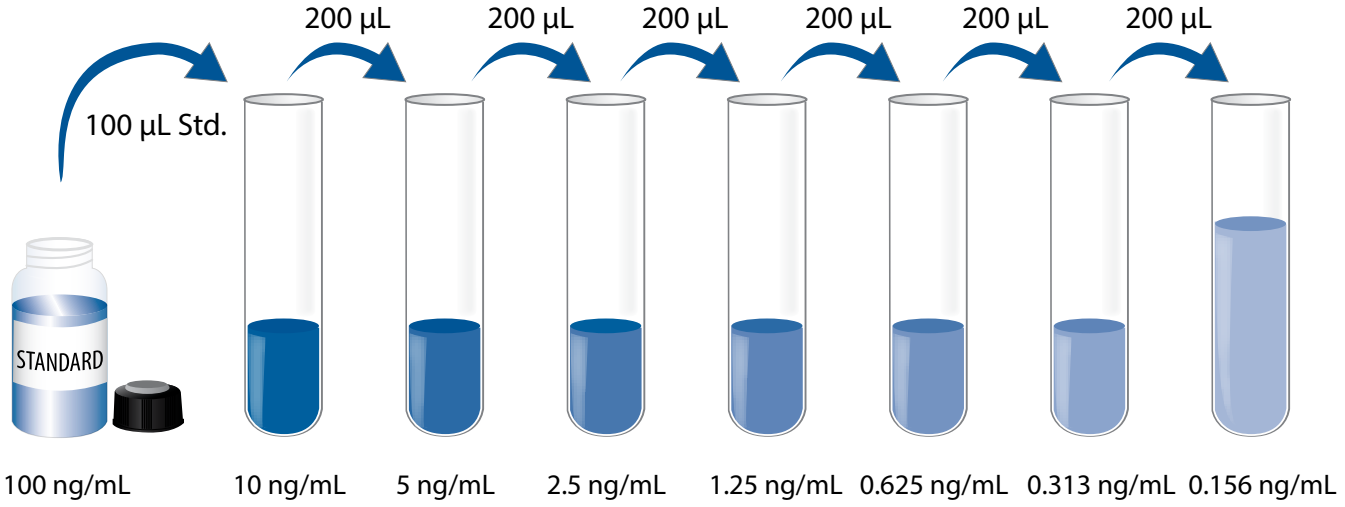
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD5-24 (1:10) - Add 20 mL of Calibrator Diluent RD5-24 concentrate to 180 mL of deionized or distilled water to prepare 200 mL of Calibrator Diluent RD5-24 (1:10).

Mouse MSP Standard - Reconstitute the Mouse MSP Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5-24 (1:10) into the 10 ng/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. Calibrator Diluent RD5-24 (1:10) 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, 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 100 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.
4. Aspirate each well and wash, repeating the process three times for a total of four 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.
5. Add 100 µL of Mouse MSP Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
6. Repeat the aspiration/wash as in step 5.
7. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
8. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. 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

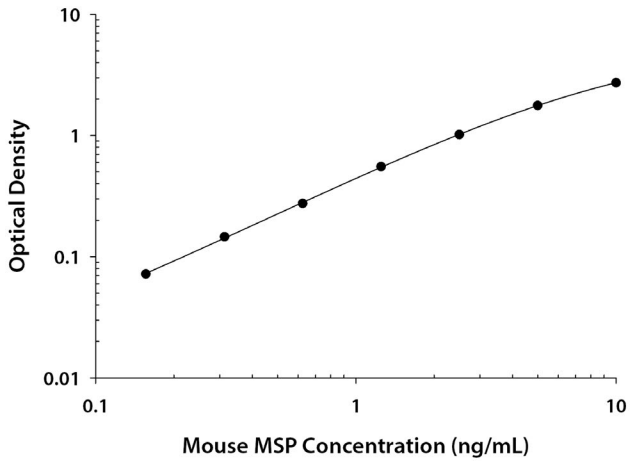
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse MSP 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	0.013 0.014	0.014	—
0.156	0.085 0.087	0.086	0.072
0.313	0.159 0.161	0.160	0.146
0.625	0.285 0.292	0.289	0.275
1.25	0.563 0.570	0.567	0.553
2.5	1.027 1.039	1.033	1.019
5	1.774 1.792	1.783	1.769
10	2.733 2.745	2.739	2.725

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 (ng/mL)	0.504	1.28	3.98	0.470	1.24	3.74
Standard deviation	0.027	0.068	0.154	0.015	0.044	0.228
CV (%)	5.4	5.3	3.9	3.2	3.6	6.1

RECOVERY

The recovery of mouse MSP spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture samples (n=4)	98	90-108%
Tissue lyates (n=4)	101	90-109%
Urine (n=4)	96	88-104%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse MSP were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay.

		Cell culture samples (n=4)	Tissue lysates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine (n=4)
1:2	Average % of Expected	100	102	99	99	99	96
	Range (%)	98-103	98-107	92-106	96-103	97-105	93-100
1:4	Average % of Expected	105	107	98	95	97	101
	Range (%)	103-108	102-117	87-111	80-107	88-108	97-107
1:8	Average % of Expected	108	110	98	94	98	110
	Range (%)	107-110	106-117	86-106	80-109	92-106	105-118
1:16	Average % of Expected	111	114	94	94	98	110
	Range (%)	109-114	107-117	85-103	81-118	92-110	104-115

SENSITIVITY

Fifty-two assays were evaluated and the minimum detectable dose (MDD) of mouse MSP ranged from 0.003-0.019 ng/mL. The mean MDD was 0.007 ng/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 CHO cell-derived disulfide-linked heterodimer recombinant mouse MSP produced at R&D Systems.

SAMPLE VALUES

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

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=10)	1781	948-2456	449
EDTA plasma (n=5)	2054	1480-2560	441
Heparin plasma (n=5)	1784	1224-2132	347

ND-Non-detectable

Sample Type	Mean of Detectable (ng/mL)	% Detectable	Range (ng/mL)
Urine (n=5)	0.674	80	ND-0.976

Cell Culture Supernates - Livers from mice were rinsed with PBS and kept on ice. Tissue was homogenized with a tissue homogenizer and cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 24 hours. An aliquot of the cell culture supernate was removed, assayed for mouse MSP, and measured 3.20 ng/mL.

Tissue Lysates - Livers from mice were rinsed with PBS, cut into 1-2 mm pieces, and homogenized with a tissue homogenizer in PBS. An equal volume of Cell Lysis Buffer 1 was added and tissues were lysed at room temperature for 30 minutes with gentle agitation. Debris was then removed by centrifugation. An aliquot of the lysate was removed, assayed for mouse MSP, and measured 152 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse single chain proform and mature heterodimer MSP.

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

Recombinant mouse:	Recombinant human:
HGF	HGF
HGF Activator	HNF-4α/NR2A1
IL-9 R	

Recombinant human MSP and recombinant human MSP (Cys672Ala) cross-react approximately 0.4% in this assay.

Recombinant mouse MSP R interferes at concentrations > 20 ng/mL.

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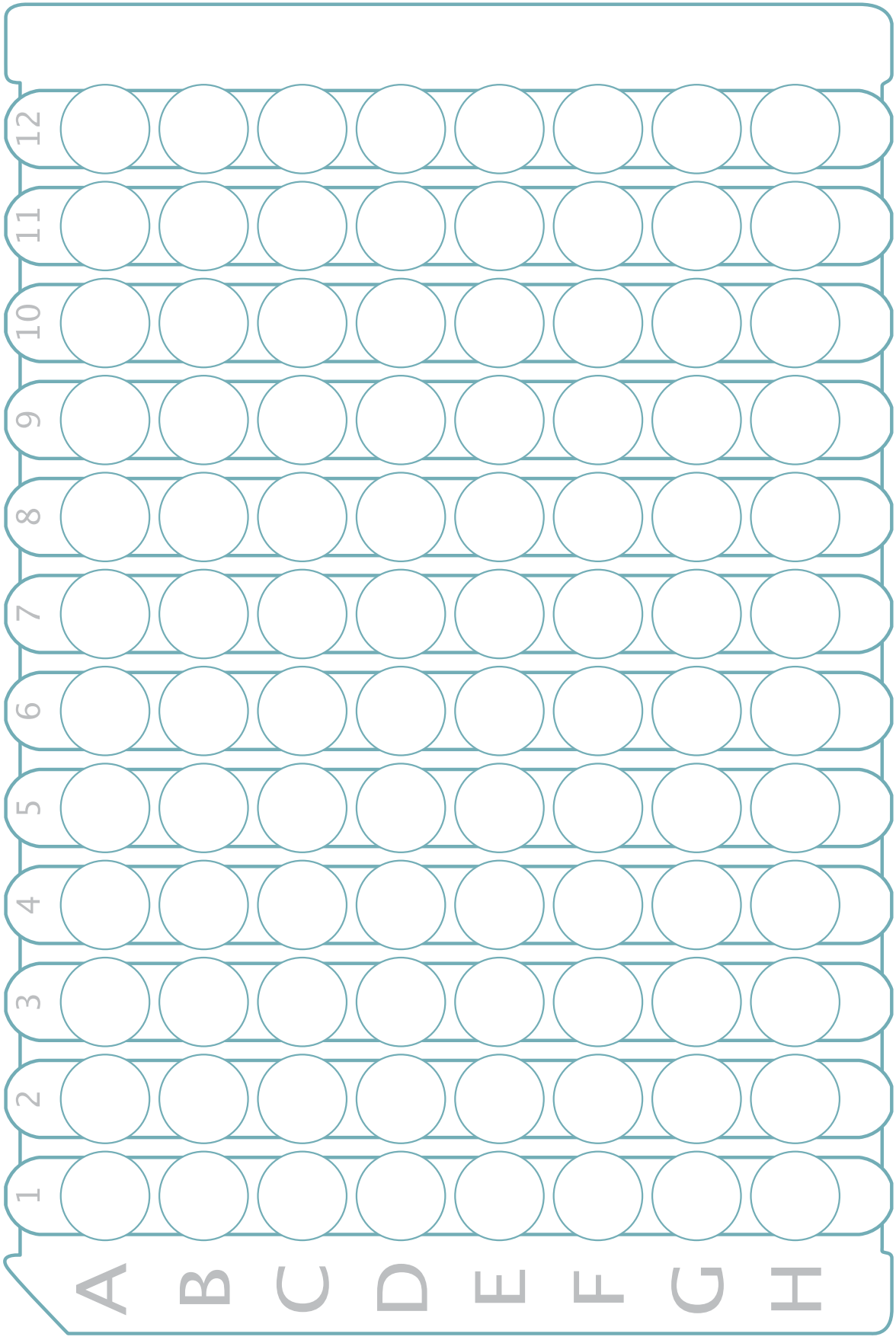
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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