Quantikine® ELISA

Mouse Pro-MMP-9 Immunoassay

Catalog Number MMP900B

For the quantitative determination of mouse Pro-Matrix Metalloproteinase 9 (Pro-MMP-9) concentrations in cell culture supernates, serum, and platelet-poor 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 the extracellular matrix (ECM) and in the processing of a variety of molecules in different subcellular environments. They play an important role in many normal physiological processes such as embryonic development, morphogenesis, reproduction, and tissue remodeling (1, 2). They also participate in inflammatory and autoimmune disorders such as arthritis, cancer, and cardiovascular disease (3-5). 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, α2-Macroglobulin, and tissue inhibitors of metalloproteinases (TIMPs) (6).

MMP-9 (also referred to as gelatinase B, 92 kDa type IV collagenase, 92 kDa gelatinase, and type V collagenase) is secreted as a glycosylated proenzyme (6-8). Activation of the proenzyme involves proteolytic removal of the N-terminal pro region, resulting in the 82 kDa active enzyme (9, 10). Mouse Pro-MMP-9 shares 72% and 82% amino acid sequence identity with human and rat MMP-9, respectively. In addition to the zinc-binding site, the catalytic domain also contains three contiguous fibronectin type II homology units responsible for binding gelatin (11). A proline-rich hinge region links the catalytic domain to the C-terminal hemopexin-like domain. *In vitro* treatment of the proenzyme with 4-aminophenylmercuric acetate (APMA) produces not only the active enzyme but also a C-terminal truncated form with activity comparable to that of the active form (12). MMP-9 degrades components of the ECM with high specific activity for denatured collagens (gelatin). It can cleave native collagens of type III, IV, V, and XI, as well as Elastin, Nidogen-1, and Vitronectin (2, 3). MMP-9 can also cleave a variety of chemokines and growth factors (e.g. IL-1β, CXCL8/IL-8, CXCL7, CXCL4, CXCL1, Latent TGF-β, membrane bound TNF-α, VEGF, and FGF basic), Amyloid β peptide, Substance P, and Myelin Basic Protein (3, 13-15). This action can increase or decrease the biological activity of soluble factors and can also liberate them from association with the ECM (16, 17). MMP-9 can also trigger signaling through various transmembrane proteins or inhibit signaling by inducing their shedding from the cell surface (e.g. CD44, E-Cadherin, Integrins, ICAM-1, and IL-2 Rα) (3, 18-20).

MMP-9 is produced by a variety of normal and transformed cells including neutrophils, monocytes, macrophages, astrocytes, fibroblasts, osteoclasts, chondrocytes, keratinocytes, endothelial and epithelial cells. It exerts physiological and pathological angiogenic and remodeling effects on the vasculature (21-25). Activated neutrophils release proMMP-9 which is free of TIMP-1, allowing the liberation of pro-angiogenic FGF-2 from the ECM (17). MMP-9 in complex with TIMP-1 does not induce FGF-2 release (17). Neutrophil-derived MMP-9 exacerbates the inflammatory response, in part by generating collagen-derived peptides that induce the release of additional neutrophil MMP-9 (26). MMP-9 also plays a role in bone formation and remodeling (1, 21, 27), methamphetamine-induced behavioral sensitization and reward (28), the regulation of neuronal synapse remodeling (29), trophoblast invasion during implantation (30), and the inactivation of Serpin α1-Proteinase Inhibitor (31). The shedding of adhesion proteins by MMP-9 has a direct effect on tumor cell invasiveness (18-20).

Circulating levels of MMP-9 are increased in many inflammatory disorders including intraluminal thrombus formation (32), atherosclerosis (33), Crohn's disease (34), hepatitis C virus infection (35), colorectal cancer (36), and Duchenne muscular dystrophy (37). The ratio of MMP-9 to TIMP-1 is also increased in multiple sclerosis serum (38) and cystic fibrosis sputum (39), but it is decreased in the serum during cytomegalovirus infection (40). Levels of free MMP-9 and complexes of MMP-9 with Lipocalin-2/NGAL are elevated in the urine of ovarian cancer and uterine tract infection patients, respectively (41, 42).

The Quantikine® Mouse Pro-MMP-9 Immunoassay is a 4.5 hour solid phase ELISA designed to measure free and TIMP-1 bound mouse Pro-MMP-9 in cell culture supernates, serum, and platelet-poor plasma. It contains NS0-expressed recombinant mouse Pro-MMP-9 and antibodies raised against the recombinant factor. Natural mouse Pro-MMP-9 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 mouse Pro-MMP-9.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse Pro-MMP-9 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any Pro-MMP-9 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse Pro-MMP-9 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 Pro-MMP-9 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.
- If samples generated values lower than the lowest standard, decrease the dilution 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.
- For best results, pipette reagents and samples into the center of each well.
- 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse Pro-MMP-9 Microplate	893186	96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibody specific for mouse Pro-MMP-9.	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 Pro-MMP-9 Standard	893728	2 vials of recombinant mouse Pro-MMP-9 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a fresh standard and control for each assay. Discard after use.
Mouse Pro-MMP-9 Control	893729	2 vials of recombinant mouse Pro-MMP-9 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse Pro-MMP-9 Conjugate	893727	12 mL of a monoclonal antibody specific for mouse Pro-MMP-9 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-34	895265	11 mL of a buffered protein base with blue dye and preservatives.	
Calibrator Diluent RD5-10	895266	2 vials (21 mL/vial) of a buffered protein solution 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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Refrigerator (for incubation at 2-8 °C).
- 500 mL graduated cylinder.
- **Polypropylene** 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 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.

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.

Platelet-poor Plasma - To measure circulating Pro-MMP-9 levels, platelet-poor plasma is recommended. Collect plasma on ice using EDTA or heparin as an anticoagulant. Centrifuge at 2-8 °C for 15 minutes at 1000 x g within 30 minutes of collection. For complete platelet removal, an additional centrifugation step is recommended. Centrifuge the separated plasma at 2-8 °C for 10 minutes at 10,000 x g. 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.*

SAMPLE PREPARATION

Cell culture supernate samples require a 2-fold dilution. A suggested 2-fold dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD5-10.

Serum samples require a 100-fold dilution. A suggested dilution is 10 µL of sample + 90 µL of Calibrator Diluent RD5-10. Complete the 100-fold dilution by adding 20 µL of the diluted sample to 180 µL of Calibrator Diluent RD5-10.

Platelet-poor plasma samples require a 20-fold dilution. A suggested 20-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent RD5-10.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

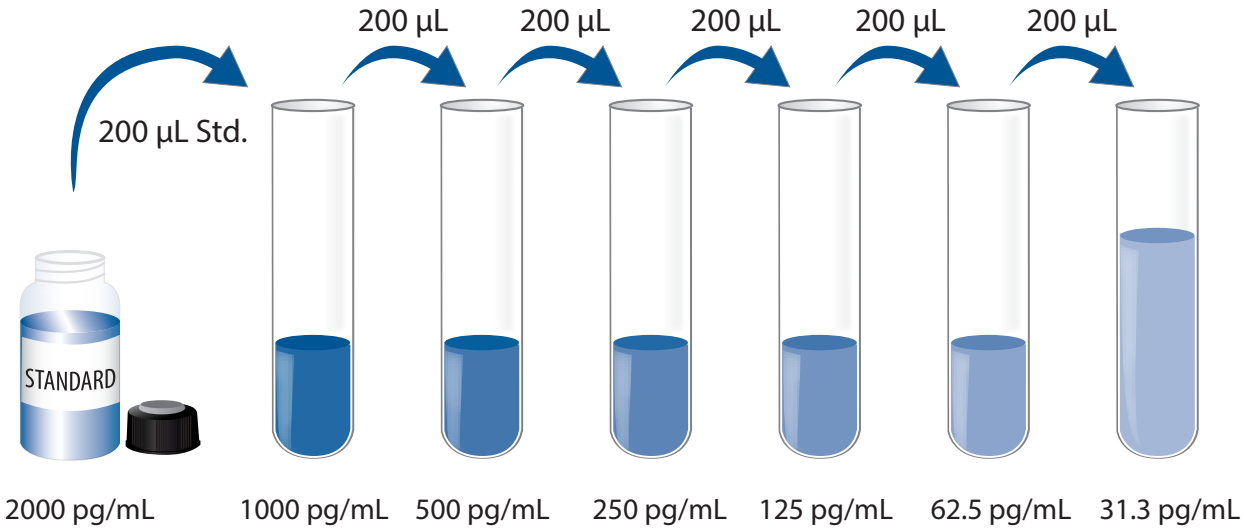
Mouse Pro-MMP-9 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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.

Mouse Pro-MMP-9 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse Pro-MMP-9 Standard with Calibrator Diluent RD5-10. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-10 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse Pro-MMP-9 Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD5-10 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 reagents and standard dilutions as directed in the previous section.
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 RD1-34 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 standards and samples assayed.
5. 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.
6. Add 100 µL of Mouse Pro-MMP-9 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours **at 2-8 °C with no agitation.**
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. If 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 the Sample Preparation section.

CALCULATION OF RESULTS

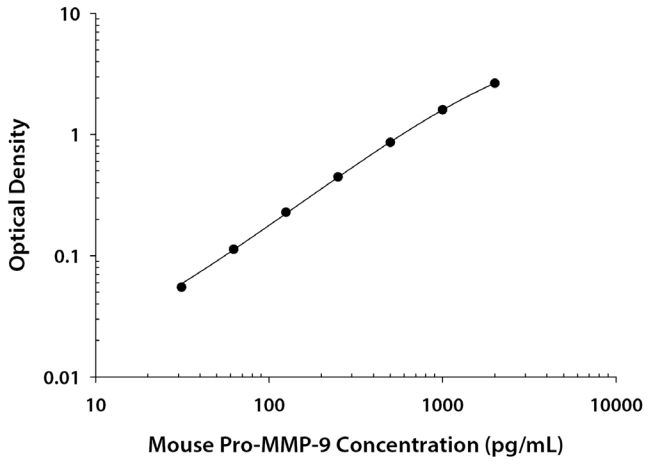
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 Pro-MMP-9 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.



(pg/mL)	O.D.	Average	Corrected
0	0.032 0.034	0.033	—
31.3	0.087 0.088	0.088	0.055
62.5	0.143 0.148	0.146	0.113
125	0.258 0.264	0.261	0.228
250	0.478 0.482	0.480	0.447
500	0.889 0.898	0.894	0.861
1000	1.589 1.674	1.632	1.599
2000	2.645 2.719	2.682	2.649

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 kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	126	206	1063	133	230	1073
Standard deviation	3.36	7.53	28.6	11.0	17.5	55.2
CV (%)	2.7	3.7	2.7	8.3	7.6	5.1

RECOVERY

The recovery of mouse Pro-MMP-9 spiked to three levels throughout the range of the assay was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

Sample Type	Average % Recovery	Range
Cell culture samples (n=4)	101	82-117%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse Pro-MMP-9 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay as directed in the Sample Preparation section.

		Cell culture supernates (n=4)	Serum (n=4)	Platelet-poor	
				EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	102	103	102	103
	Range (%)	99-107	101-107	97-107	101-106
1:4	Average % of Expected	106	105	105	107
	Range (%)	103-112	102-111	99-110	105-111
1:8	Average % of Expected	108	109	110	108
	Range (%)	104-113	104-114	105-114	107-108
1:16	Average % of Expected	107	109	108	107
	Range (%)	100-110	106-113	105-115	100-112

SENSITIVITY

Forty-two assays were evaluated and the minimum detectable dose (MDD) of mouse Pro-MMP-9 ranged from 0.92-14.0 pg/mL. The mean MDD was 3.01 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 mouse Pro-MMP-9 produced at R&D Systems®.

SAMPLE VALUES

Serum/Platelet-poor Plasma - Samples were evaluated for the presence of mouse Pro-MMP-9 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=20)	113	27.0-263	66.3
Platelet-poor heparin plasma (n=20)	10.1	5.50-32.4	6.58
Platelet-poor EDTA plasma (n=20)	8.51	1.81-28.1	5.82

Cell Culture Supernates:

J774A.1 mouse reticulum cell sarcoma macrophage cells were seeded at 1 x 10^6 cells/mL and cultured for 3 days in DMEM supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for mouse Pro-MMP-9, and measured 485,700 pg/mL.

3T3-L1 mouse embryonic fibroblast adipose-like cells were seeded at 1 x 10^6 cells/mL and grown in DMEM supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 4 days until confluent. An aliquot of the cell culture supernate was removed, assayed for mouse Pro-MMP-9, and measured 255 pg/mL.

NR6R-3T3 mouse fibroblasts were seeded at 1 x 10^4 cells/mL and grown for 4 days in DMEM supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for mouse Pro-MMP-9, and measured 1042 pg/mL.

P388D1 mouse lymphoma cells were seeded at 7.5 x 10^4 cells/mL and grown for 3 days in RPMI supplemented with 10% fetal bovine serum and 2 mM L-glutamine. An aliquot of the cell culture supernate was removed, assayed for mouse Pro-MMP-9, and measured 3628 pg/mL.

IC-21 mouse macrophage cells were seeded at 5 x 10^4 cells/mL and grown for 3 days in RPMI supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol and 2 mM L-glutamine. An aliquot of the cell culture supernate was removed, assayed for mouse Pro-MMP-9, and measured 248 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse Pro-MMP-9, even when complexed with TIMP-1, but does not recognize active mouse MMP-9.

The factors listed below were prepared at 200 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 200 ng/mL in a mid-range mouse Pro-MMP-9 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

Lipocalin-2
MMP-2
MMP-3
MMP-7
MMP-8
MMP-12
TIMP-1

Recombinant rat:

Lipocalin-2
MMP-8
MMP-9
TIMP-1

Recombinant human:

Lipocalin-1
Lipocalin-2
MMP-1
MMP-2
MMP-3
MMP-7
MMP-8
MMP-9
MMP-9/Lipocalin-2
MMP-9/TIMP-1
MMP-10
MMP-12 (Hemopexin domain)
Pro-MMP-12
MMP-13
MMP-14
Pro-MMP-16
TIMP-2
TIMP-3
TIMP-4

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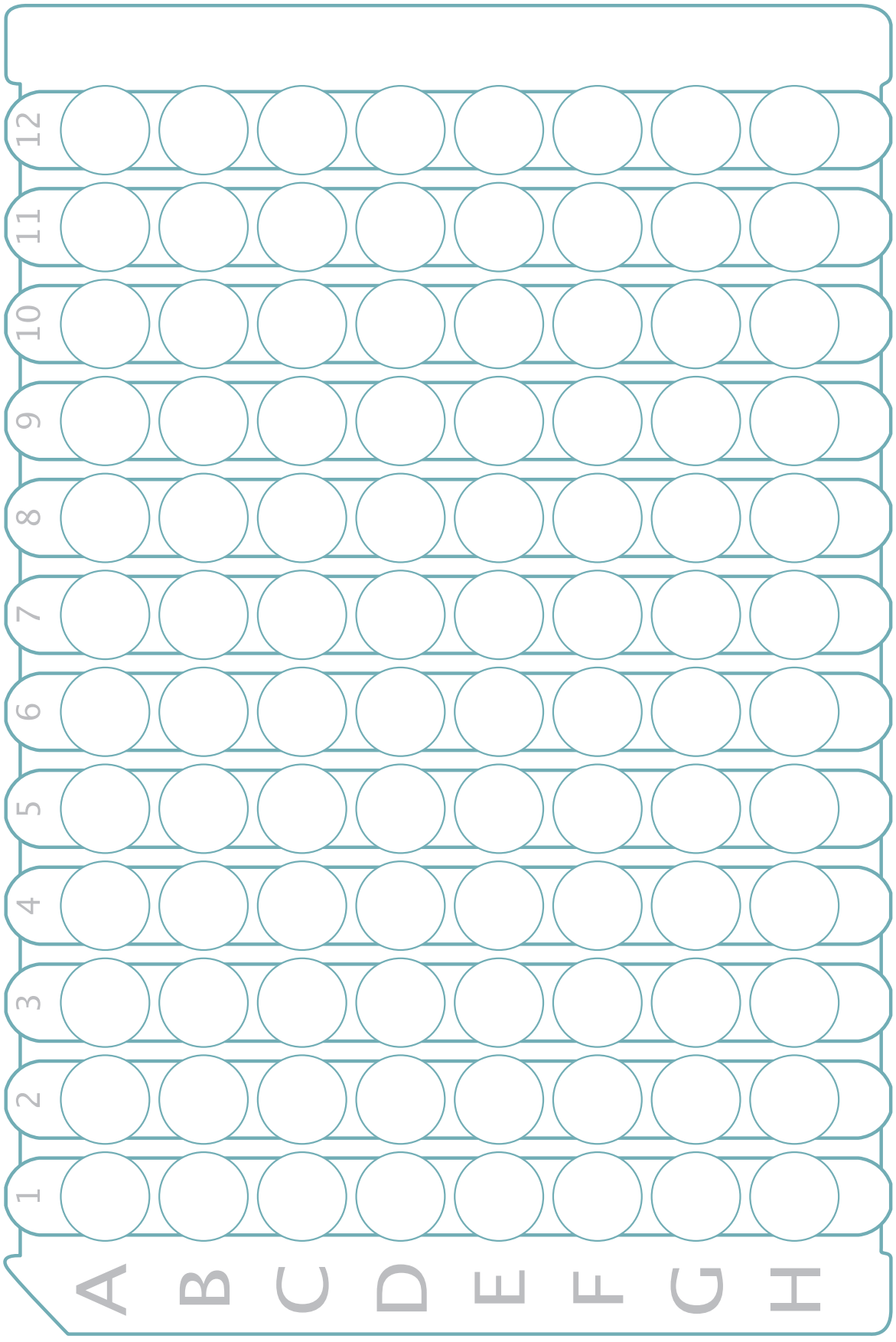
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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