Quantikine® ELISA

Human Total MMP-8 Immunoassay

Catalog Number DMP800B

For the quantitative determination of human active and pro-Matrix Metalloproteinase 8 (Total MMP-8) concentrations in cell culture supernates, tissue lysates, serum, plasma, platelet-poor 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), also called matrixins, constitute a family of zinc and calcium dependent endopeptidases that function in the breakdown of extracellular matrix (ECM). 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 and the inhibition of active enzymes by endogenous inhibitors, α_2-macroglobulins and tissue inhibitors of metalloproteinases (TIMPs).

MMP-8 (also referred to as neutrophil collagenase and collagenase 2) is stored intracellularly as a latent proenzyme in the specific granules of polymorphonuclear leukocytes. PMNs play an essential role in phagocytosis and also have a high capacity to infiltrate connective tissue. Various agents, such as IL-1 and IL-8, TNF-α, and GM-CSF, stimulate the release of MMP-8 from neutrophils, a key enzyme initiating the breakdown of the ECM, especially during pathological processes such as inflammation, rheumatoid arthritis or osteoarthritis (3).

MMP-8 consists of several domains. The N-terminal pro-domain contains the cysteine switch motif conserved in MMPs that maintains MMP-8 in the latent state (4). Activation of the proenzyme occurs in the extracellular space after secretion, resulting in the removal of the pro-domain. MMP-8 activation can be achieved *in vitro* by proteases such as itself, MMP-3, MMP-10, and cathepsin G, and by chemicals such as mercury compounds and oxygen radicals (3). The resulting active enzyme consists of a catalytic domain with a zinc-binding motif conserved in metzincins (5,6). A short hinge peptide links the catalytic domain to the C-terminal hemopexin-like domain. The active MMP-8 is capable of cleaving types I, II and III triple-helical collagen into the characteristic ¾ and ¼ fragments. MMP-8 can not only activate but also degrade itself. In addition, the active MMP-8 can cleave natural proteins such as fibronectin, cartilage aggrecan, serpins, and peptides such as angiotensin and substance P (3).

The Quantikine® Human Total MMP-8 Immunoassay is a 4.5 hour solid phase ELISA designed to measure total MMP-8 (pro- and active MMP-8) in cell culture supernates, tissue lysates, serum, plasma, platelet-poor plasma, and saliva. It contains NS0-expressed recombinant human MMP-8, and antibodies raised against the recombinant protein. Natural human MMP-8 showed dose-response 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 human MMP-8.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human MMP-8 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MMP-8 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human MMP-8 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 MMP-8 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 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.
- 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 #	DESCRIPTION	STORAGE OF OPENED/RECONSTITUTED MATERIAL
Human MMP-8 Microplate	899007	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human MMP-8.	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 MMP-8 Standard	899009	2 vials (100 ng/vial) of recombinant human MMP-8 in a buffered protein base with preservatives; lyophilized.	Discard after use. Use a fresh standard for each assay.
Human MMP-8 Conjugate	899008	21 mL of a monoclonal antibody specific for human MMP-8 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-125	896332	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P	895151	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	895032	6 mL of 2 N sulfuric 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.
- 500 mL graduated cylinder.
- 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.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human Total MMP-8 Controls (optional; R&D Systems®, Catalog # QC251).

SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES

- RIPA Buffer with protease inhibitors

PRECAUTIONS

MMP-8 is detectable in saliva. Take precautionary measures 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.

Tissue Lysates - Lysates were prepared as described in the Sample Value section.

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.

Platelet-poor Plasma - 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 of the separated plasma at 1500 x g for 10 minutes at 2-8 °C is recommended. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

MMP-8 is present in platelet granules and is released upon platelet activation. Therefore, to measure circulating levels of MMP-8, platelet-poor plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical and Laboratory Standards Institute (CLSI), result in incomplete removal of platelets from blood.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly hemolyzed are not suitable for ise in this assay.

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Use polypropylene tubes.

Plasma samples may require a 2-fold dilution prior to assay due to high endogenous values. A suggested 2-fold dilution is 150 µL of sample + 150 µL of Calibrator Diluent RD5P (diluted 1:5)*.
Note: *Platelet-poor plasma samples do not require dilution.*

Serum and saliva samples require at least a 10-fold dilution prior to assay due to high endogenous values. A suggested 10-fold dilution is 30 µL of sample + 270 µL of Calibrator Diluent RD5P (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High concentrations of MMP-8 are found in saliva. The use of a face mask and gloves is recommended to protect kit reagents from contamination.*

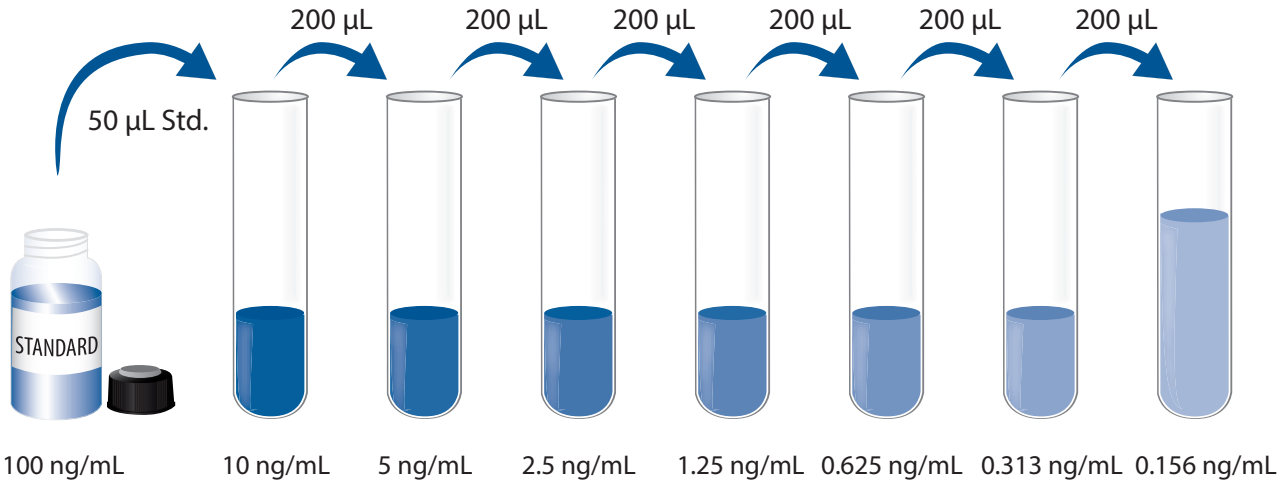
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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 10 mL of Calibrator Diluent RD5P to 40 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent RD5P (diluted 1:5).

Human Total MMP-8 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Total MMP-8 Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 450 µL of Calibrator Diluent RD5-P (diluted 1:5) into the 10 ng/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. 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 concentrations of MMP-8 are found in saliva. The use of a face mask and gloves is recommended to protect kit reagents from contamination.*

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 50 µL of Assay Diluent RD1-125 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 200 µL of Human Total MMP-8 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 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 may require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

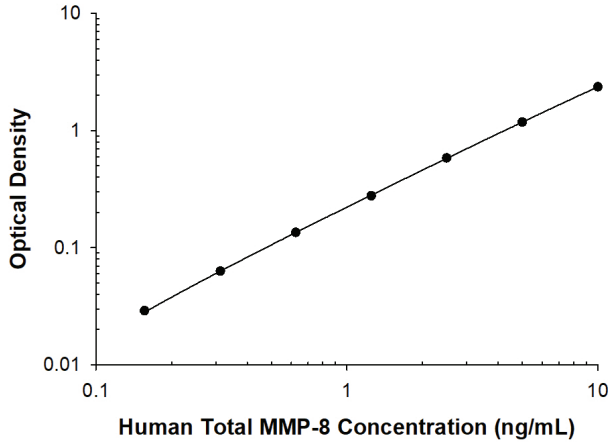
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human Total MMP-8 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.



(ng/mL)	O.D.	Average	Corrected
0	0.003 0.004	0.004	—
0.156	0.028 0.029	0.029	0.025
0.313	0.062 0.064	0.063	0.059
0.625	0.134 0.135	0.135	0.131
1.25	0.275 0.280	0.278	0.274
2.5	0.580 0.590	0.585	0.581
5	1.181 1.183	1.182	1.178
10	2.321 2.408	2.365	2.361

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)	0.764	2.38	4.55	0.836	2.41	5.07
Standard deviation	0.049	0.070	0.299	0.055	0.171	0.270
CV (%)	6.4	2.9	6.6	6.6	7.1	5.3

RECOVERY

The recovery of human Total MMP-8 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	98	93-103%
Serum* (n=4)	101	93-109%
Extraction buffer (n=2)	84	83-85%
EDTA plasma* (n=4)	92	86-99%
Heparin plasma* (n=4)	92	86-99%
Platelet-poor EDTA plasma (n=4)	85	79-91%
Platelet-poor heparin plasma (n=4)	84	78-89%
Saliva* (n=4)	96	86-111%

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

SENSITIVITY

Thirty-two assays were evaluated and the minimum detectable dose (MDD) of human Total MMP-8 ranged from 0.004-0.058 ng/mL. The mean MDD was 0.013 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.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Total MMP-8 in various matrices were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates* (n=4)	Tissue lysates (n=2)	Serum* (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	98	87	98	102	102
	Range (%)	96-102	85-89	95-100	100-104	100-103
1:4	Average % of Expected	94	99	96	100	104
	Range (%)	93-95	98-101	93-102	94-108	103-107
1:8	Average % of Expected	94	103	97	100	104
	Range (%)	92-97	100-105	91-104	95-109	101-108
1:16	Average % of Expected	94	100	95	101	104
	Range (%)	90-98	97-103	85-100	88-115	100-108

		Platelet-poor		
		EDTA plasma (n=4)	Heparin plasma (n=4)	Saliva* (n=4)
1:2	Average % of Expected	104	109	98
	Range (%)	100-109	101-116	95-101
1:4	Average % of Expected	109	110	98
	Range (%)	105-111	104-117	98-98
1:8	Average % of Expected	108	112	96
	Range (%)	105-114	105-122	93-101
1:16	Average % of Expected	110	114	92
	Range (%)	103-116	106-122	88-98

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

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human MMP-8 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Platelet-poor Plasma/Saliva - Samples from apparently healthy volunteers were evaluated for the presence of human Total MMP-8 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=30)	22.3	4.67-76.5	14.6
EDTA plasma (n=30)	2.68	0.863-5.57	1.14
Heparin plasma (n=30)	5.92	1.03-19.7	4.43
Platelet-poor EDTA plasma (n=30)	1.42	0.506-3.39	0.670
Platelet-poor heparin plasma (n=30)	1.07	0.467-2.25	0.442
Saliva (n=20)	69.1	2.08-314	78.1

Cell Culture Supernates:
Human polymorphonuclear cells were cultured for 4 days in RPMI 1640 supplemented with 10% fetal bovine serum, 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 human Total MMP-8, and measured 186 ng/mL.

Human peripheral blood mononuclear cells were cultured in serum-free RPMI 1640 supplemented with 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were stimulated with 10 µg/mL PHA for 24 hours. An aliquot of the cell culture supernate was removed, assayed for human Total MMP-8, and measured 41.6 ng/mL.

SK-Mel-28 human malignant melanoma cells were cultured in MEM supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until confluent. An aliquot of the cell culture supernate was removed, assayed for human Total MMP-8, and measured at 37.3 ng/mL.

Tissue Lysates - Human placenta was rinsed with PBS, cut into 1-2 mm pieces, and homogenized with a tissue homogenizer in PBS. An equal volume of RIPA buffer containing protease inhibitors was added and tissues were lysed on ice for 30 minutes with gentle agitation. Debris was then removed by centrifugation. A BCA was done on the supernate to determine total protein concentration. 50 µg of the tissue lysate was removed, assayed for human Total MMP-8, and measured 3.17 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human MMP-8.

The factors listed below were prepared at 100 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 100 ng/mL in a mid-range recombinant human MMP-8 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ADAM8
ADAM9
ADAM10
ADAM12
ADAM15
ADAM19
ADAM22
ADAM23
ADAM28
ADAM33
ADAMTS1
ADAMTS4
ADAMTS5
ADAMTS13
ADAMTS15
Lipocalin-2
MMP-1
MMP-2
MMP-3
MMP-7
MMP-9
MMP-10
MMP-12
MMP-13
TACE
TIMP-1
TIMP-2
TIMP-3
TIMP-4

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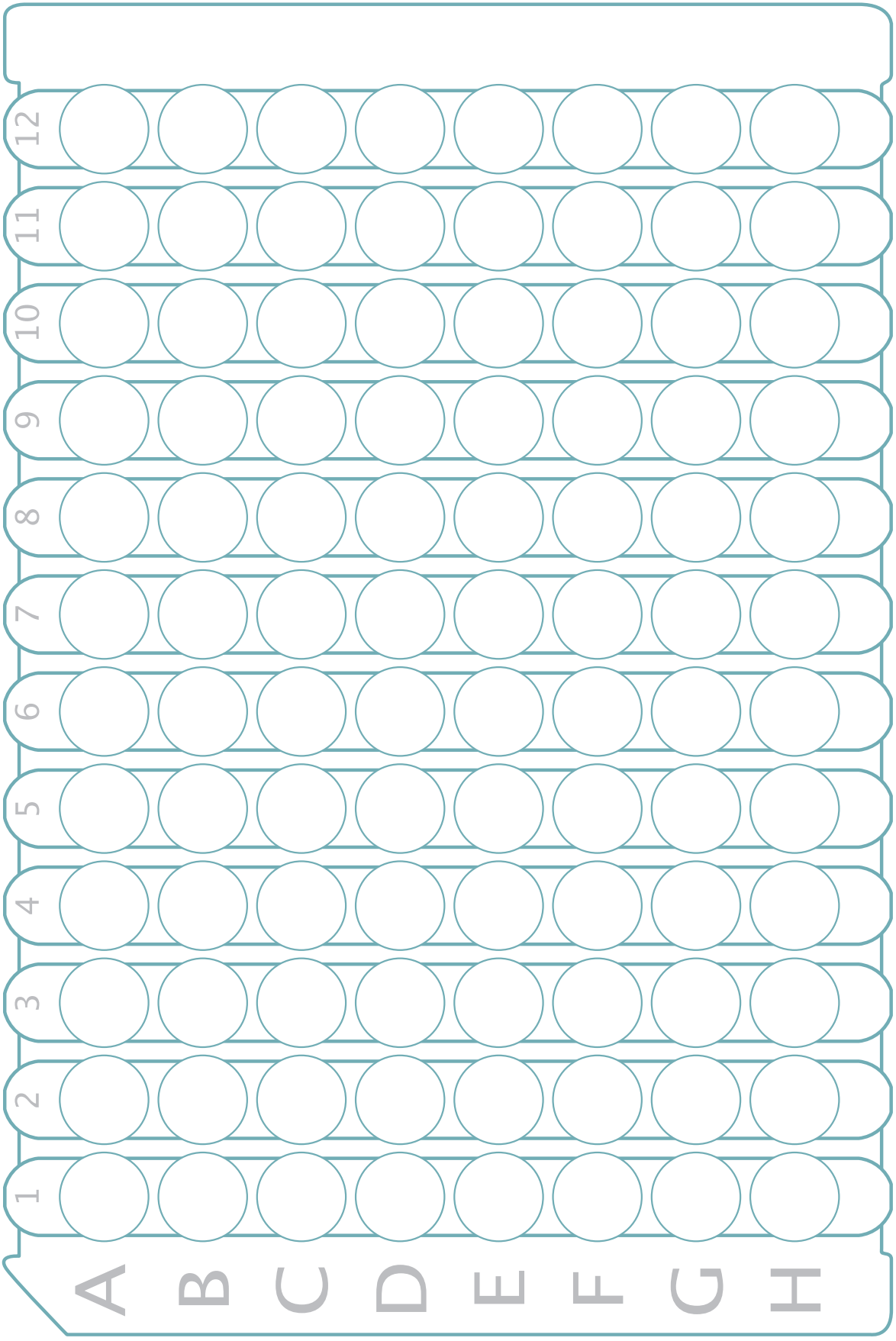
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES