Quantikine® ELISA

Mouse CCL4/MIP-1β Immunoassay

Catalog Number MMB00

For the quantitative determination of mouse Macrophage Inflammatory Protein 1 beta (MIP-1β) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. Read this package insert in its entirety before using this product.

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 Inflammatory Protein 1 alpha and beta (MIP-1α and MIP-1β) were originally co-purified from medium conditioned by an LPS-stimulated mouse macrophage cell line (1). These two closely related but distinct proteins are members of the CC subfamily of chemokines. Chemokines comprise a large superfamily of small, inducible, secreted cytokines that are involved in a variety of immune and inflammatory responses. Additional chemokine subfamilies, defined by the arrangement of the conserved cysteine (C) residues include the C, CXC, and CX3C subfamilies (2).

Mouse MIP-1β cDNA encodes a 92 amino acid (aa) residue precursor protein with a 23 aa residue signal peptide that is cleaved to generate the 69 aa residue secreted mature protein (1-3). Mature mouse MIP-1β shares approximately 68% and 67% aa identity with mature mouse MIP-1α and MIP-1γ, respectively (1, 3-6). Mouse MIP-1β also shares 85% and 77% aa sequence identity with rat and human MIP-1β, respectively (1, 7-9). MIP-1β expression can be induced in a variety of mouse cell types, including mast cells (10), endothelial cells (11), macrophages (12, 13), $CD8^+$ T cells (14), and intra-epithelial γδ-T cells (15).

The biological activities of MIP-1β overlap with those of other β chemokines. MIP-1β is a monocyte chemoattractant (2) and has been reported to selectively recruit T-lymphocytes to lymph nodes (10) and other sites of inflammation (16). MIP-1β has also been shown to enhance macrophage effector functions by inducing NO synthesis in parasite-infected macrophages (13).

Chemokines mediate their activities by binding to target cell surface chemokine receptors that belong to the large family of G protein-coupled, seven transmembrane domain receptors (17). To date, at least nine signaling CC chemokine receptors (CCR1 through CCR9) have been cloned and characterized (17-24). Many of these chemokine receptors have overlapping ligand specificities. Three of these receptors, CCR1, 5, and 8 have been shown to mediate the biological activities of MIP-1β (18-22).

The Quantikine® Mouse CCL4/MIP-1β Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse MIP-1β levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse MIP-1β and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse MIP-1β accurately. Results obtained using natural mouse MIP-1β 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 mouse MIP-1β.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An affinity purified polyclonal antibody specific for mouse MIP-1β has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any MIP-1β present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse MIP-1β 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 MIP-1β 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.
- For best results, pipette reagents and samples into the center of each well.
- 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 MIP-1β Microplate	890757	96 well microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse MIP-1β.	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 MIP-1β Standard	890758	Recombinant mouse MIP-1β in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C.* Avoid repeated freeze-thaw cycles.
Mouse MIP-1β Control	890058	Recombinant mouse MIP-1β in a buffered protein base with preservatives; lyophilized. The assayed value of the control should be within the range specified on the label.	
Mouse MIP-1β Conjugate	890760	12 mL of a polyclonal antibody specific for mouse MIP-1β conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-41	895514	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-16	895302	21 mL of diluted animal serum 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.
- 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.

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 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

Cell culture supernate samples may require dilution.

Serum and plasma samples require a 2-fold dilution prior to assay. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD5-16.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

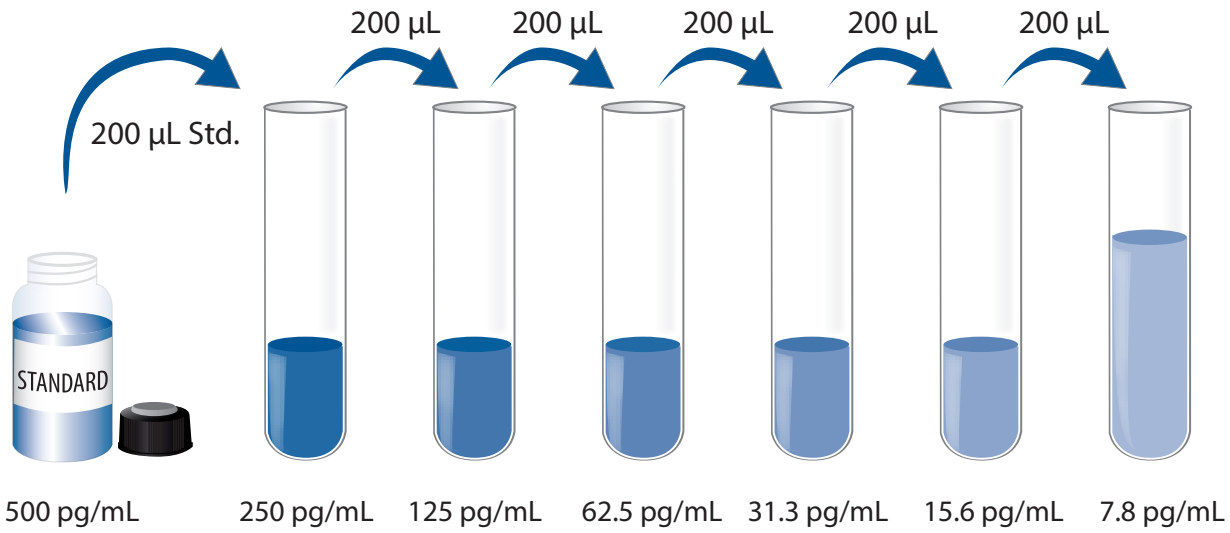
Mouse MIP-1β Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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 MIP-1β Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse MIP-1β Standard with Calibrator Diluent RD5-16. Do not substitute other diluents. This reconstitution produces a stock solution of 500 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 RD5-16 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse MIP-1β Standard (500 pg/mL) serves as the high standard. Calibrator Diluent RD5-16 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, 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 RD1-41 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 Mouse MIP-1β 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 substraction 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 Sample Preparation section.

CALCULATION OF RESULTS

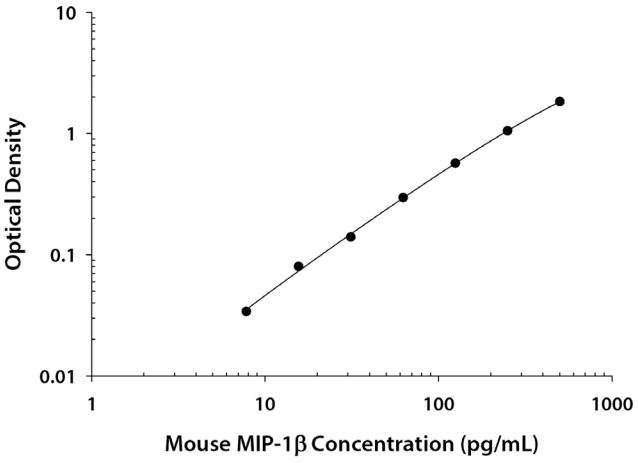
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 MIP-1β 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	0.052 0.057	0.054	—
7.8	0.088 0.089	0.088	0.034
15.6	0.131 0.136	0.134	0.080
31.3	0.192 0.197	0.194	0.140
62.5	0.346 0.354	0.350	0.296
125	0.620 0.627	0.624	0.570
250	1.102 1.109	1.106	1.052
500	1.880 1.894	1.887	1.833

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 (pg/mL)	23.1	73	234	23.7	77	245
Standard deviation	2.2	4.0	5.4	1.7	3.4	12.5
CV (%)	9.5	5.5	2.3	7.2	4.4	5.1

RECOVERY

The recovery of mouse MIP-1β spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernate (n=5)	104	86-118%
Serum (n=5)	101	93-109%
EDTA plasma (n=5)	99	87-106%

SENSITIVITY

The minimum detectable dose (MDD) of mouse MIP-1β is typically less than 3 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 *E. coli*-expressed recombinant mouse MIP-1β produced at R&D Systems®.

LINEARITY

To assess the linearity of the assay, five or more samples containing and/or spiked with high concentrations of mouse MIP-1β were diluted with calibrator diluent and assayed. Results from typical sample dilutions are shown.

Samples	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Cell culture supernates	Neat	266	———	———
	1:2	126	133	95%
	1:4	60	66	91%
	1:8	31	33	94%
	1:16	16	16	100%
Serum*	Neat	224	———	———
	1:2	112	112	100%
	1:4	56	56	100%
	1:8	27	28	96%
	1:16	13	14	93%
EDTA plasma*	Neat	251	———	———
	1:2	127	126	101%
	1:4	65	63	103%
	1:8	34	32	106%
	1:16	17	16	106%

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

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse MIP-1β in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=40)	40.3	18.1-79.6	14.5
EDTA plasma (n=5)	30.7	16.1-50.5	15.6

Cell Culture Supernates - J774A.1 mouse reticulum cell sarcoma macrophage cells (1 x 10^6 cells/mL) were cultured for 2 days in RPMI containing 10% fetal bovine serum, 500 ng/mL recombinant mouse IFN-γ, and stimulated with 1 µg/mL LPS. An aliquot of the cell culture supernate was removed, assayed for mouse MIP-1β, and measured 26.3 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse MIP-1β.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse MIP-1β control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C10
CCR1
CCR5
CCR8
Eotaxin
G-CSF
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-10 R
IL-12 p70
IL-13
IL-17
IL-18
JE/MCP-1
KC
Leptin
LIF
MARC
M-CSF
MCP-5
MIP-1α
MIP-2
OSM
RANTES
SCF
TNF-α
TNF RI
TNF RII
Tpo
VEGF

Recombinant human:

GROα
GROβ
IL-8
MIP-1α
MIP-1β

Rat serum samples and various rat cell culture supernates were tested for potential cross-reactivity in this kit. All values were non-detectable.

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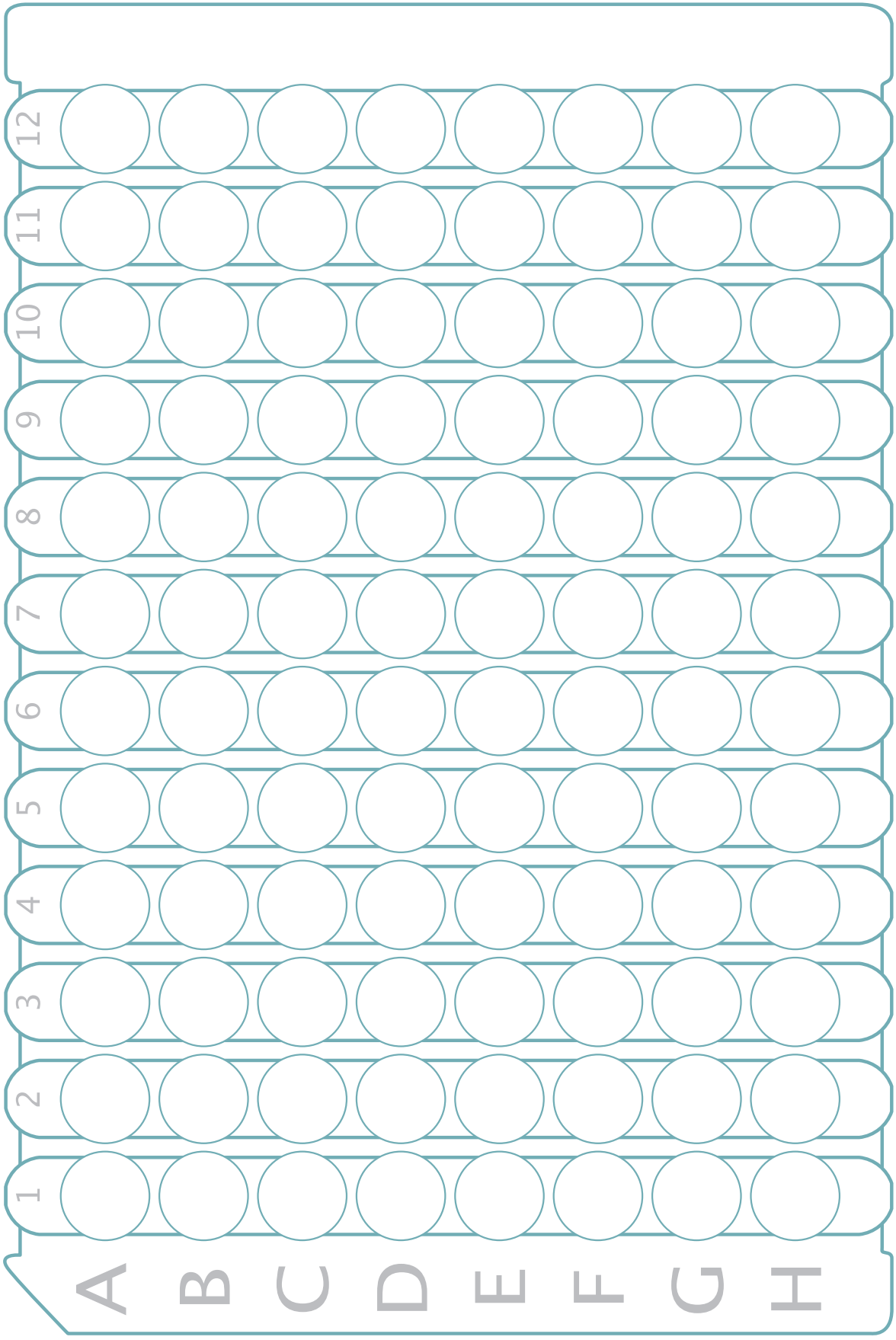
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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