Quantikine® ELISA

Mouse/Rat CCL2/JE/MCP-1 Immunoassay

Catalog Number MJE00
SMJE00
PMJE00

For the quantitative determination of mouse or rat Monocyte Chemotactic Protein 1 (MCP-1) concentrations in cell culture supernates and serum.

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

The mouse JE gene was originally described as a platelet-derived growth factor-inducible gene in mouse fibroblasts (1). The protein encoded by mouse JE was found to belong to the large CC chemokine family of inflammatory and immunoregulatory cytokines. Among CC chemokine family members, JE is functionally and structurally most closely related to the MCP/eotaxin subfamily of proteins. Within this MCP/eotaxin subfamily, five human (MCP-1, 2, 3, 4 and eotaxin) and four mouse (JE/MCP-1, MARC/MCP-3, MCP-5, and eotaxin) proteins have been identified (1-3). At the amino acid (aa) sequence level, mature human MCP-1 shows 55%, 59%, and 66% identity with the analogous regions of mouse JE, MARC, and MCP-5, respectively (1-3). Although JE has been presumed to be the mouse homolog of human MCP-1 (3-6), the more recently isolated mouse MCP-5 is actually more homologous and may be considered to be a second human MCP-1 homolog (7).

Mouse MCP-1 cDNA encodes a 148 aa residue precursor protein with a predicted 23 aa residue signal peptide that is cleaved to generate a putative mature protein of 125 aa residues (1-3). Compared to mature human MCP-1, mouse MCP-1 has a 49 aa residue carboxy-terminal extension that is rich in serine and threonine residues. Recombinant MCP-1 expressed in CHO cells (2) as well as natural MCP-1 purified from mouse astrocytes (4, 10) and a mouse thymic epithelial cell line (8), were shown to be approximately 30 kDa glycoproteins with multiple O-linked oligosaccharide chains added to the 49 aa residue C-terminal domain. Nonetheless, the natural form of MCP-1 produced by virus-stimulated mouse L929 fibroblasts occurs as a non-glycosylated 7-8 kDa protein that lacks the C-terminal domain (12). The carboxy-terminal domain has been found not to be required for mouse MCP-1 activity. Besides fibroblasts, astrocytes and epithelial cells, mouse MCP-1 has been found to be expressed in macrophages (4, 9), mast cells (7), endothelial cells (7), osteoblasts and ameloblasts (11). The expression of mouse MCP-1 is induced after stimulation with inflammatory stimuli including viruses, LPS, and cytokines such as TNF-α, IL-1, IFN-γ, and PDGF (1, 3, 4, 12, 13).

Mouse MCP-1 is a potent chemoattractant for monocytes/macrophages and lymphocytes (3, 4, 13, 17). It has also been shown to be involved in the regulation of Th1/Th2 lymphocyte differentiation, enhancing Th2 development by increasing IL-4 production and inhibiting IL-12 production (18-21). The activities of mouse MCP-1 have been shown to be mediated by the mouse CC chemokine receptor CCR2, a G protein-coupled, seven transmembrane domain receptor (6, 14, 15). Mouse CCR2 cDNA encodes a 373 aa residue protein that shows the highest (80%) overall identity at the aa sequence level with the human MCP-1 receptor, CCR2B (14-16). The gene for mouse CCR2 has been mapped to mouse chromosome 9, in close proximity with mouse CCR1 and CCR3. High levels of mouse CCR2 expression have been detected in monocytes/macrophages.

The Quantikine Mouse/Rat CCL2/JE/MCP-1 immunoassay is a 4.5 hour solid-phase ELISA designed to measure MCP-1 in mouse or rat cell culture supernates and serum. It contains *E. coli*-expressed recombinant mouse MCP-1 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant mouse MCP-1. Results obtained using natural mouse or rat MCP-1 showed dose response curves that were parallel to the standard curves obtained using the Quantikine mouse kit standards. These results indicate that this kit can be used to determine relative mass values for natural MCP-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse/rat MCP-1 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any MCP-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse/rat MCP-1 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 MCP-1 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 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 #	CATALOG # MJE00	CATALOG # SMJE00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse/Rat MCP-1 Microplate	890601	2 plates	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with polyclonal antibody specific for mouse/rat MCP-1.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse/Rat MCP-1 Standard	890603	1 vial	3 vials	Recombinant mouse MCP-1 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.*
Mouse/Rat MCP-1 Control	890604	1 vial	3 vials	Recombinant mouse MCP-1 in a buffered protein base with preservatives; lyophilized. The assayed value of the Control should be within the range specified on the label.	
Mouse/Rat MCP-1 Conjugate	890602	1 vial	3 vials	23 mL/vial of a polyclonal antibody against mouse/rat MCP-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895038	1 vial	3 vials	12 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-3	895436	2 vials	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials	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	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	8 strips	24 strips	Adhesive strips.	

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

MJE00 contains sufficient materials to run ELISAs on two 96 well plates.
SMJE00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PMJE00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

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.
- 1000 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 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 and 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.

Note: *Grossly hemolyzed or lipemic samples are not suitable for use in this assay.*

SAMPLE PREPARATION

Mouse cell culture supernate and serum samples require a 2-fold dilution into Calibrator Diluent RD5-3. A suggested dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD5-3.

Rat serum samples require a 4-fold dilution into Calibration Diluent RD5-3. A suggested dilution is 40 µL of sample + 120 µL of Calibrator Diluent RD5-3.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

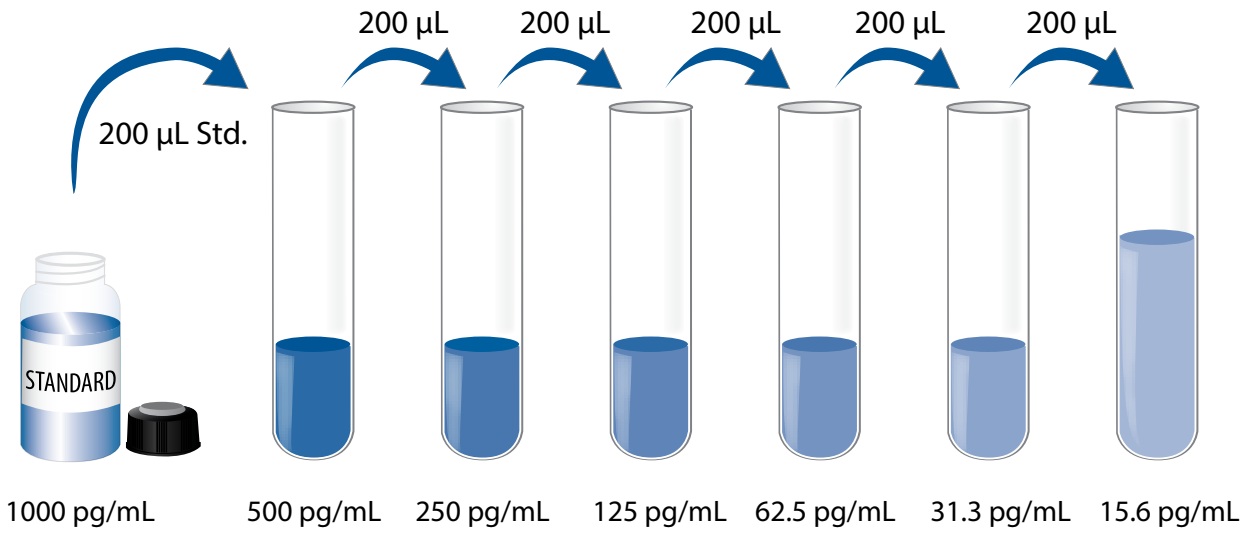
Mouse/Rat MCP-1 Control - Reconstitute the Control with 1.0 mL deionized or distilled water. Assay the Control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, 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/Rat MCP-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse/Rat MCP-1 Standard with Calibrator Diluent RD5-3. Do not substitute other diluents. This reconstitution produces a stock solution of 1000 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 RD5-3 into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse/Rat MCP-1 Standard serves as the high standard (1000 pg/mL). Calibrator Diluent RD5-3 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 samples, standards, and control be assayed in duplicate.

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 RD1W 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/Rat MCP-1 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 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

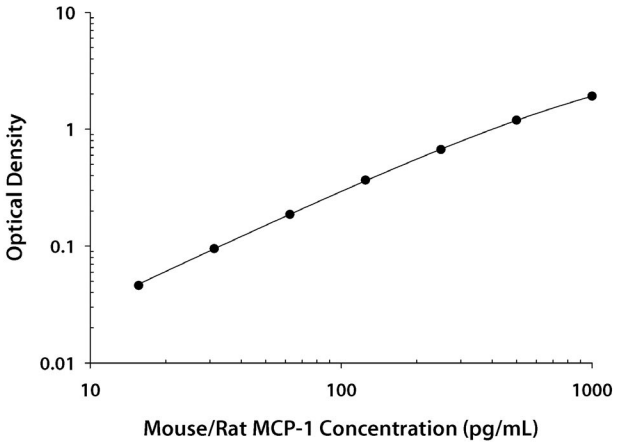
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse/rat MCP-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.

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	0.009 0.009	0.009	—
15.6	0.054 0.056	0.055	0.046
31.3	0.100 0.107	0.104	0.095
62.5	0.187 0.204	0.196	0.187
125	0.362 0.388	0.375	0.366
250	0.649 0.707	0.678	0.669
500	1.172 1.230	1.201	1.192
1000	1.909 1.941	1.925	1.916

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	47.1	311	768	43.6	310	783
Standard deviation	3.9	15.8	40.6	3.2	14.3	42.8
CV (%)	8.3	5.1	5.3	7.3	4.6	5.5

RECOVERY

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

Sample Type	Average % Recovery	Range
Mouse cell culture supernates* (n=6)	104	91-119%
Mouse serum* (n=9)	99	89-113%
Rat cell culture supernates (n=4)	109	103-115%
Rat serum* (n=4)	109	103-116%

*Samples were 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 various concentrations of mouse MCP-1 in each matrix, diluted with Calibrator Diluent and then assayed.

		Mouse		Rat	
		Cell culture samples* (n=4)	Serum* (n=4)	Cell culture samples (n=2)	Serum* (n=3)
1:2	Average % of Expected	103	109	100	101
	Range (%)	101-106	104-115	96-104	94-105
1:4	Average % of Expected	100	109	98	103
	Range (%)	97-103	104-114	95-100	92-112
1:8	Average % of Expected	100	109	99	102
	Range (%)	96-103	105-115	93-104	92-109
1:16	Average % of Expected	95	108	98	102
	Range (%)	92-100	103-112	92-104	94-112

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

SENSITIVITY

The minimum detectable dose (MDD) of MCP-1 is typically less than 2 pg/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 *E. coli*-expressed recombinant mouse MCP-1 produced at R&D Systems.

SAMPLE VALUES

Serum - Mouse and rat samples were evaluated for the presence of MCP-1 in this assay.

	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Mouse serum* (n=40)	130	50-294	47
Rat serum* (n=10)	1346	768-1584	278

*Samples were diluted prior to assay.

Cell Culture Supernates:
Mouse heart tissue was cut into 1-2 mm pieces and cultured in 10 mL of RPMI supplemented with 10% fetal bovine serum for 7 days. An aliquot of the cell culture supernate was removed, assayed for levels of mouse MCP-1, and measured 3000 pg/mL.

L-929 mouse fibroblasts (1 x 10^5 cells/mL) were cultured for 3 days in MEM containing L-glutamine and 10% equine serum. An aliquot of the cell culture supernate was removed, assayed for levels of mouse MCP-1 and measured 13,000 pg/mL.

Rat splenocytes were cultured for 22 hours in RPMI supplemented with 10% fetal bovine serum. The cells were stimulated with 10 µg/mL of PHA and 10 ng/mL of PMA. An aliquot of the cell culture supernate was removed, assayed for levels of rat MCP-1 and measured 891 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse and rat MCP-1.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-3 and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse/rat MCP-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

CCL3/MIP-1α
CCL4/MIP-1β
CCL5/RANTES
CCL6/C10
CCL7/MCP-3/MARC
CCL11/Eotaxin
CCL12/MCP-5
CXCL1/KC
CXCL2/MIP-2
G-CSF
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-10 R
IL-12
IL-13
LIF
M-CSF
SCF
TNF-α
Tpo
VEGF

Recombinant human:

CCL2/MCP-1
CCL3/MIP-1α
CCL4/MIP-1β
CCL5/RANTES
CCL7/MCP-3
CCL8/MCP-2
CCL11/Eotaxin
CXCL1/GROα
CXCL2/GROβ
CXCL8/IL-8

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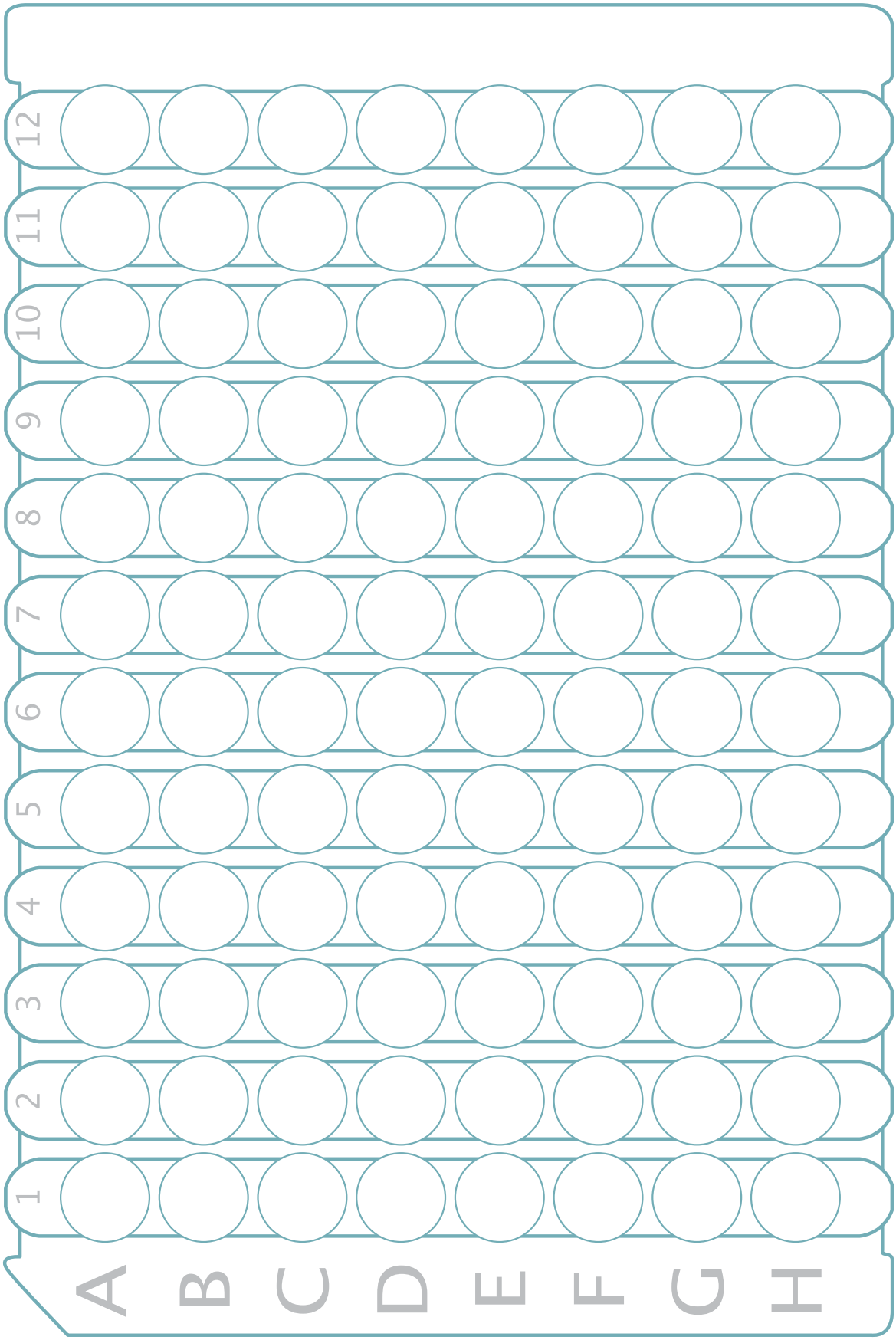
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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