Quantikine™ ELISA

Mouse M-CSF Immunoassay

Catalog Number MMC00B

For the quantitative determination of mouse Macrophage Colony Stimulating Factor (M-CSF) concentrations in cell culture supernates, serum, and 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

M-CSF, also known as CSF-1, is a four-α-helical-bundle cytokine that is the primary regulator of macrophage survival, proliferation and differentiation (1-7). M-CSF is found as isoforms of various sizes. All isoforms contain the N-terminal 150 amino acid (aa) portion that is necessary and sufficient for interaction with the M-CSF receptor, but may vary in activity and half-life (7-15). Full length mouse M-CSF transcripts encode a 520 aa type I transmembrane (TM) protein that forms a 140 kDa covalent dimer. Differential processing produces two proteolytically cleaved, secreted dimers. One is an N- and O-glycosylated 86 kDa dimer, while the other is modified by both glycosylation and chondroitin-sulfate proteoglycan (PG) to generate a 200 kDa subunit. Although PG-modified M-CSF can circulate, it may be immobilized by attachment to type V collagen (11). Shorter transcripts encode M-CSF that lacks cleavage and PG sites and produces an N-glycosylated 68 kDa TM dimer and a slowly produced 44 kDa secreted dimer (12). The region of mature mouse M-CSF that is common to all forms shares 87%, 83%, 82%, and 81% aa identity with corresponding regions of rat, canine, bovine, and human M-CSF, respectively (1, 2). Human M-CSF is active in the mouse, but mouse M-CSF is reported to be species-specific. Sources of M-CSF include fibroblasts, activated macrophages, endometrial secretory epithelium, bone marrow stromal cells, vitamin D-stimulated osteoblasts, and activated endothelial cells (3-8, 16).

The M-CSF receptor (M-CSF R, also called c-*fms*) transduces the pleotropic effects of M-CSF and mediates its endocytosis. Engagement of M-CSF dimers by M-CSF R induces receptor dimerization, followed by phosphorylation at multiple sites (4, 14, 17, 18). M-CSF R is expressed on monocytes and tissue macrophages, and treatment with M-CSF promotes differentiation of macrophages, kidney mesangial cells, liver Kupffer cells, brain microglial cells, bone osteoclasts, fetal trophoblast cells, skin Langerhans cells, intestinal Paneth cells, and blood and lymph node plasmacytoid dendritic cells (3-5, 8, 19). M-CSF R is also expressed on osteoblasts where it downregulates RANKL production, thus allowing M-CSF to limit osteoclast production (20). IL-34 can also engage the M-CSF R, but downstream effects may differ (21).

M-CSF is essential for macrophage-related functions such as bone resorption, vascular development, and innate immunity. M-CSF-deficient (op/op) mice are deficient in macrophages and are osteopetrotic due to insufficient differentiation of bone-resorbing osteoclasts (3, 6-8, 20, 22). They also show failure of teeth to erupt, infertility, and defects in development of nervous, vascular and lymphatic systems (4, 7, 16, 22). M-CSF regulates the release of cytokines and other inflammatory modulators from macrophages, and stimulates chemotaxis and pinocytosis (4, 5, 7). Circulating M-CSF increases during pregnancy and supports implantation and growth of the decidua and placenta (3, 7, 16). M-CSF expression can also be increased during infection or in inflammatory disorders such as inflammatory bowel disease (3, 5, 6). Both M-CSF and its receptor can be expressed by a number of cancers, allowing M-CSF to act as an autocrine growth factor for cancer cells. Macrophages can also be recruited to tumor tissues, supplying M-CSF as a paracrine growth factor (23). On the other hand, M-CSF can have anti-cancer effects by priming and enhancing macrophage killing of tumor cells and microorganisms (3). It is thought that expression of M-CSF R on cancer cells facilitates metastasis to the bone by chemotaxis toward osteoblast-produced M-CSF and by promoting osteolysis (3, 24).

INTRODUCTION *CONTINUED*

The Quantikine™ Mouse M-CSF Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse M-CSF levels in cell culture supernates, serum, and plasma. It contains HEK293-expressed recombinant mouse M-CSF and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant mouse M-CSF. Results obtained using natural mouse M-CSF 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 M-CSF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse M-CSF has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any M-CSF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse M-CSF 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 M-CSF 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 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.

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 SDS on our website prior to use.

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 M-CSF Microplate	899265	96 well microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse M-CSF.	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 M-CSF Standard	899267	2 vials of recombinant mouse M-CSF in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard and control for each assay. Discard after use.
Mouse M-CSF Control	899268	2 vials of recombinant mouse M-CSF in a buffered protein base with preservatives; lyophilized. The assayed value of the control should be within the range specified on the label.	
Mouse M-CSF Conjugate	899266	12 mL of a monoclonal antibody specific for mouse M-CSF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-38	895301	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P	895151	21 mL vial of a buffered protein solution with preservatives. *Use diluted 1:5 in this assay.*	
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
- 100 mL and 500 mL graduated cylinders
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards and samples

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 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.*

SAMPLE PREPARATION

Due to high endogenous levels, a 30-fold dilution is recommended for mouse serum, EDTA plasma, and heparin plasma.

A suggested 30-fold dilution is 10 µL sample + 290 µL Calibrator Diluent RD5P (diluted 1:5)*.

Cell culture supernates may require dilution due to high endogenous level.

Multiple dilutions are recommended for unknown samples.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

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

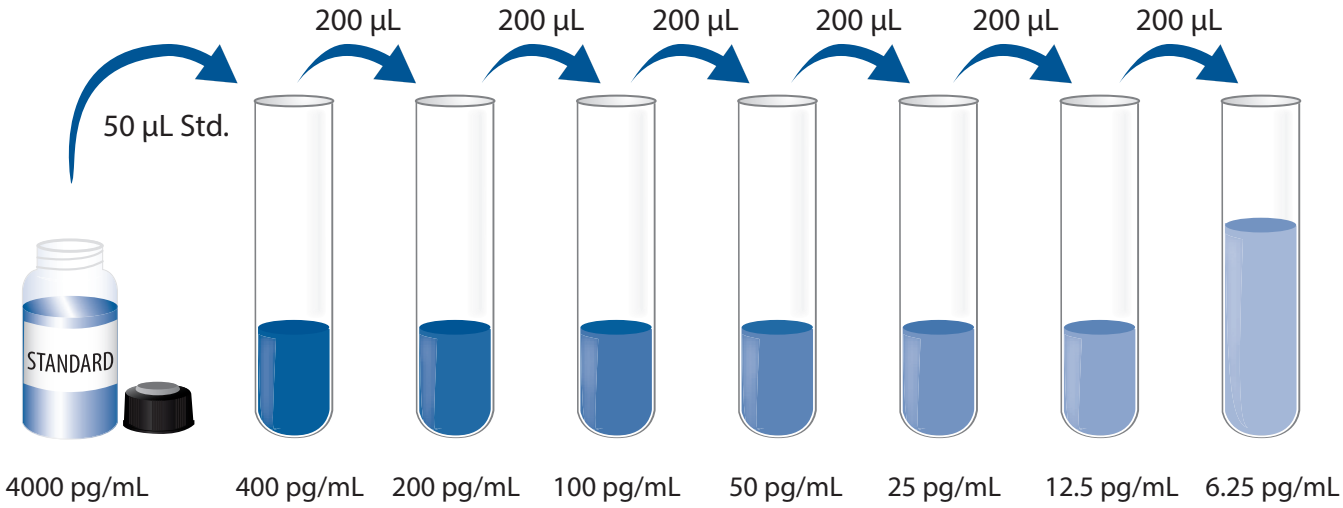
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 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).

Mouse M-CSF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse M-CSF Standard with deionized or distilled water. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5P (diluted 1:5) into the 400 pg/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 400 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) 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 RD1-38 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 M-CSF 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 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. 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 Sample Preparation section.

CALCULATION OF RESULTS

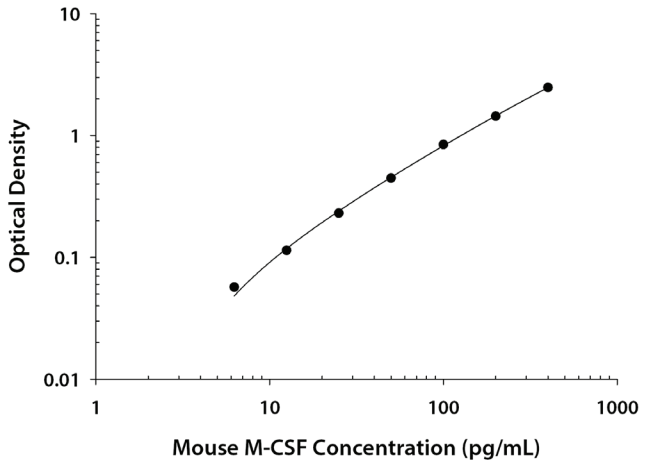
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 M-CSF 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	0.010 0.011	0.011	—
6.25	0.057 0.057	0.057	0.046
12.5	0.113 0.114	0.114	0.103
25	0.227 0.235	0.231	0.220
50	0.434 0.462	0.448	0.437
100	0.832 0.858	0.845	0.834
200	1.438 1.442	1.440	1.429
400	2.453 2.511	2.482	2.471

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 kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	21.8	213	57.8	23.0	224	11.2
Standard deviation	1.05	9.97	1.72	1.61	14.8	0.765
CV (%)	4.8	4.7	3.0	7.0	6.6	6.8

RECOVERY

The recovery of mouse M-CSF spiked to three levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	101	97-108%
Serum (n=4)	94	88-98%
EDTA plasma (n=4)	97	92-105%
Heparin plasma (n=4)	99	93-110%

SENSITIVITY

Twenty-two assays were evaluated and the minimum detectable dose (MDD) of mouse M-CSF ranged from 0.080-0.486 pg/mL. The mean MDD was 0.187 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 highly purified HEK293-expressed recombinant mouse M-CSF produced at R&D Systems®.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse M-CSF in each matrix were diluted with calibrator diluent and assayed.

		Cell culture supernate (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	101	104	102	104
	Range (%)	98-105	101-107	99-107	101-106
1:4	Average % of Expected	99	103	102	103
	Range (%)	96-102	101-105	97-109	98-109
1:8	Average % of Expected	103	105	104	104
	Range (%)	100-105	101-111	99-115	98-114
1:16	Average % of Expected	104	104	104	104
	Range (%)	100-107	99-108	98-115	96-114

*Samples were diluted prior to assay.

SAMPLE VALUES

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

	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=12)	1220	666-1970	420
EDTA plasma (n=7)	618	373-857	157
Heparin plasma (n=7)	619	382-839	170

Cell Culture Supernates:

RAW264.7 mouse monocyte/macrophage cells were cultured in DMEM High Glucose supplemented with 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were left untreated, treated with 1 µg/mL lipopolysaccharide (LPS), or treated with 1 µg/mL LPS and 100 ng/mL of recombinant mouse IFN-γ (R&D Systems®, Catalog # 485-MI/CF) for 48 hours. Aliquots of the cell culture supernates were removed and assayed for levels of mouse M-CSF.

J774A.1 mouse monocyte/macrophage cells were cultured in DMEM High Glucose supplemented with 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were left untreated, treated with 1 µg/mL lipopolysaccharide (LPS), or treated with 1 µg/mL LPS and 100 ng/mL of recombinant mouse IFN-γ (R&D Systems, Catalog # 485-MI/CF) for 48 hours. Aliquots of the cell culture supernates were removed and assayed for levels of mouse M-CSF.

Condition	RAW264.7 (pg/mL)	J774A.1 (pg/mL)
Unstimulated	ND	ND
Stimulated (LPS)	188	360
Stimulated (LPS and mouse IFN-γ)	238	296

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant mouse M-CSF.

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

Recombinant mouse:

Flt-3/Flk-2
Flt-3 Ligand
G-CSF
GM-CSF
M-CSF R
SCF

Recombinant human:

M-CSF

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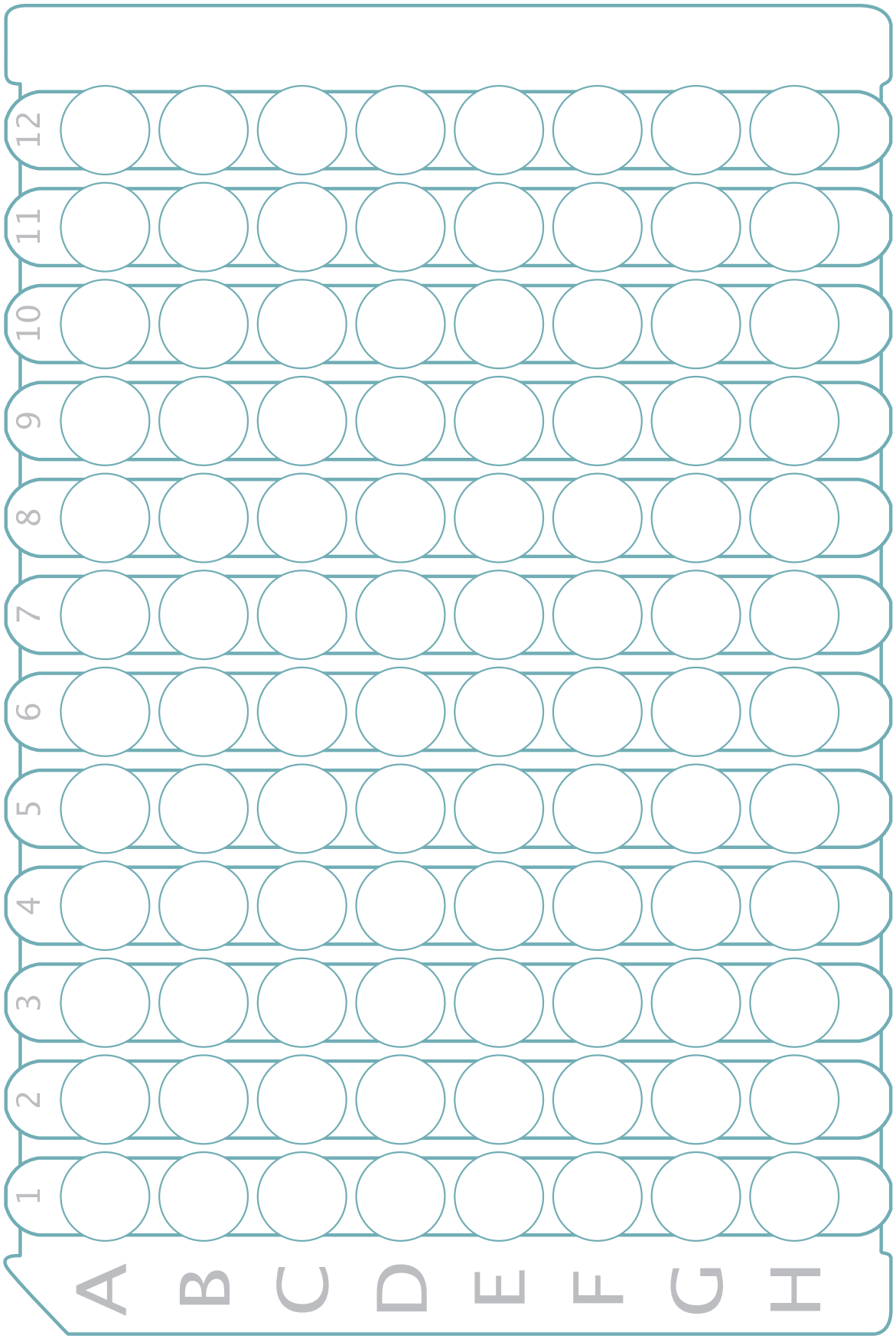
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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