Quantikine® ELISA

Mouse CCL22/MDC Immunoassay

Catalog Number MCC220

For the quantitative determination of mouse Macrophage-Derived Chemokine (MDC) 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.

TABLE OF CONTENTS

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INTRODUCTION

Mouse MDC (Macrophage-Derived Chemokine), also known as ABCD-1, is a member of the CC or β chemokine family (1-3) and is designated CCL22. It is synthesized as a 92 amino acid (aa) precursor that has a 24 aa signal sequence and a 68 aa mature segment. The 8 kDa mature mouse MDC contains four conserved cysteine residues that form two intrachain disulfide bonds, a C-terminal heparin-binding motif, and no potential N-linked glycosylation sites (4, 5). Mature mouse MDC shares 65% and 88% aa sequence identity with mature human and rat MDC, respectively (4, 6-9). Mouse MDC also shares 32% aa sequence identity with mouse CCL17/TARC, the only other chemokine that binds and activates CCR4, the sole MDC receptor identified to date (1, 2, 10-12). Chemokines are subject to amino-terminal processing, which regulates chemokine biological activity both positively and negatively. The 69 aa mature human $MDC_{1\text{-}69}$ can be processed by CD26/dipeptidyl-peptidase IV to generate N-terminally truncated human $MDC_{3\text{-}69}$ and $MDC_{5\text{-}69}$ sequentially. Truncated MDC proteins do not bind the chemokine receptor CCR4 and have markedly reduced activity on lymphocytes and monocyte-derived dendritic cells; however, they have the same activity as intact MDC on monocytes (13, 14). Based on the activity of truncated MDC on monocytes, an additional MDC receptor is implicated (2, 14). Cells known to express MDC include B cells (4, 15), microglia (16), dendritic cells and macrophages (17, 18), monocytes (18), IFN-γ-activated keratinocytes (19, 20), colonic columnar epithelium (21), $CD4^+$ T cells (18), thymic medullary epithelium (Hassal's corpuscles) (6), and activated NK cells (18, 22).

MDC plays an important role in lymphocyte migration. MDC produced by antigen-presenting dendritic cells chemoattracts CCR4-bearing activated (or memory) T cells to enhance immune responses and increase effector functions (2, 4, 18, 23, 24). Dendritic cell-derived MDC can also chemoattract $CD4^+$ $CD25^+$ $CTLA4^+$ regulatory T cells expressing CCR4 to inflammatory sites or secondary lymphoid tissues where T cell activation can be attenuated (25). B cell-derived MDC may allow for T cell-B cell interaction with the subsequent formation of germinal centers (4). MDC is a potent chemoattractant for additional cell types including dendritic cells (6, 7) thymocytes (2, 6), and activated NK cells (7, 26). Although MDC does not chemoattract eosinophils *in vitro*, it has been shown to initiate eosinophil degranulation both *in vitro* and *in vivo* (29). Additional functions reported for MDC include the activation of platelet function (27) and inhibition of synaptic transmission between hippocampal neurons (28).

The Quantikine® Mouse CCL22/MDC Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse MDC in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse MDC and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse MDC showed linear 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 MDC.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse MDC has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any MDC present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse MDC 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 MDC 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.
- 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 MDC Microplate	892548	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse MDC.	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 MDC Conjugate	892549	12 mL of a polyclonal antibody specific for mouse MDC conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse MDC Standard	892550	Recombinant mouse MDC in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse MDC Control	892551	Recombinant mouse MDC in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1-63	895352	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-16	895302	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	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.
- **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.

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.*
Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.

SAMPLE PREPARATION

Serum and plasma samples require a 3-fold dilution prior to assay. A suggested 3-fold dilution is 50 µL of sample + 100 µL of Calibrator Diluent RD5-16.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

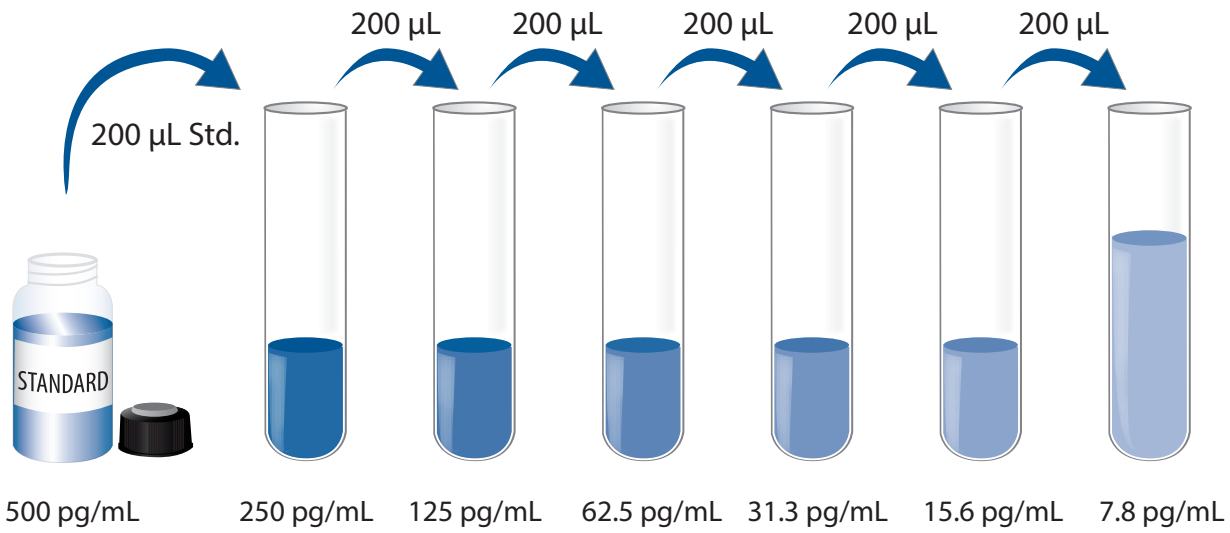
Mouse MDC Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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 MDC Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse MDC 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.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-16 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse MDC 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 all 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 RD1-63 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 MDC 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 Sample Preparation section.

CALCULATION OF RESULTS

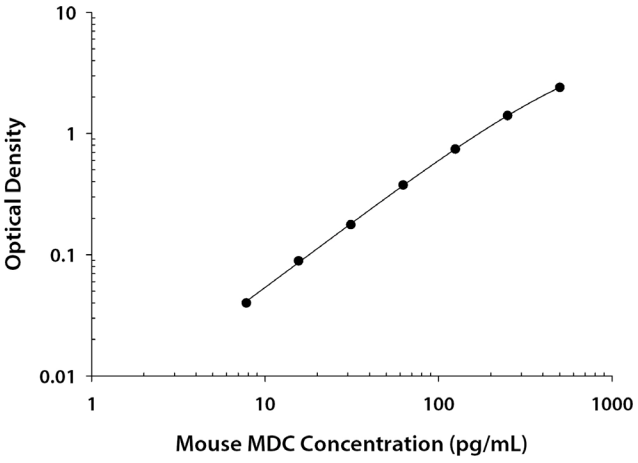
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 MDC 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 prior to assay, 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	0.016 0.019	0.018	—
7.8	0.054 0.063	0.058	0.040
15.6	0.101 0.113	0.107	0.089
31.3	0.187 0.203	0.195	0.177
62.5	0.385 0.403	0.394	0.376
125	0.747 0.774	0.760	0.742
250	1.397 1.451	1.424	1.406
500	2.408 2.436	2.422	2.404

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-four 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	24	24	24
Mean (pg/mL)	19.9	51.8	382	20.0	52.6	383
Standard deviation	1.3	2.5	19.2	1.7	2.9	28.7
CV (%)	6.5	4.8	5.0	8.5	5.5	7.5

RECOVERY

The recovery of mouse MDC spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=7)	102	87-112%
Serum* (n=5)	91	82-100%
EDTA plasma* (n=4)	94	89-98%
Heparin plasma* (n=4)	90	80-109%

*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 high concentrations of mouse MDC in each matrix were diluted with calibrator diluent and assayed.

		Cell culture supernates (n=5)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	98	106	107	105
	Range (%)	92-105	104-109	105-110	103-108
1:4	Average % of Expected	95	107	107	106
	Range (%)	89-100	104-109	104-112	103-111
1:8	Average % of Expected	96	111	109	105
	Range (%)	90-101	109-113	105-114	101-109
1:16	Average % of Expected	98	114	113	102
	Range (%)	91-106	113-117	109-117	99-104

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

SENSITIVITY

Ten assays were evaluated and the minimum detectable dose (MDD) of mouse MDC ranged from 0.6-1.8 pg/mL. The mean MDD was 1.2 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 MDC produced at R&D Systems®.

SAMPLE VALUES

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

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=20)	188	95	ND-640
EDTA plasma (n=20)	176	95	ND-255
Heparin plasma (n=20)	212	100	78-422

ND=Non-detectable

Cell Culture Supernates:

J774.A1 mouse reticulum cell sarcoma macrophage cells (1 x 10^6 cells/mL) were cultured for 3 days in DMEM supplemented with 10% fetal bovine serum, 100 ng/mL of recombinant mouse IFN-γ and 1.0 µg/mL LPS. An aliquot of the cell culture supernate was removed, assayed for mouse MDC, and measured 699 pg/mL.

Mouse spleen cells (1 x 10^6 cells/mL) were cultured for 4 days in RPMI supplemented with 10% fetal bovine serum and stimulated with 1.0 µg/mL LPS. An aliquot of the cell culture supernate was removed, assayed for mouse MDC, and measured 4727 pg/mL.

Rat splenocytes were also tested in this assay. An aliquot of the cell culture supernate was removed, assayed for mouse MDC, and measured 1123 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse MDC.

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 MDC control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

6Ckine
Eotaxin
Fractalkine
JE/MCP-1
KC
MARC
MCP-5
MIG
MIP-1α
MIP-1β
MIP-1γ
MIP-3α
MIP-2
RANTES
TARC
TECK

Recombinant human:

MDC

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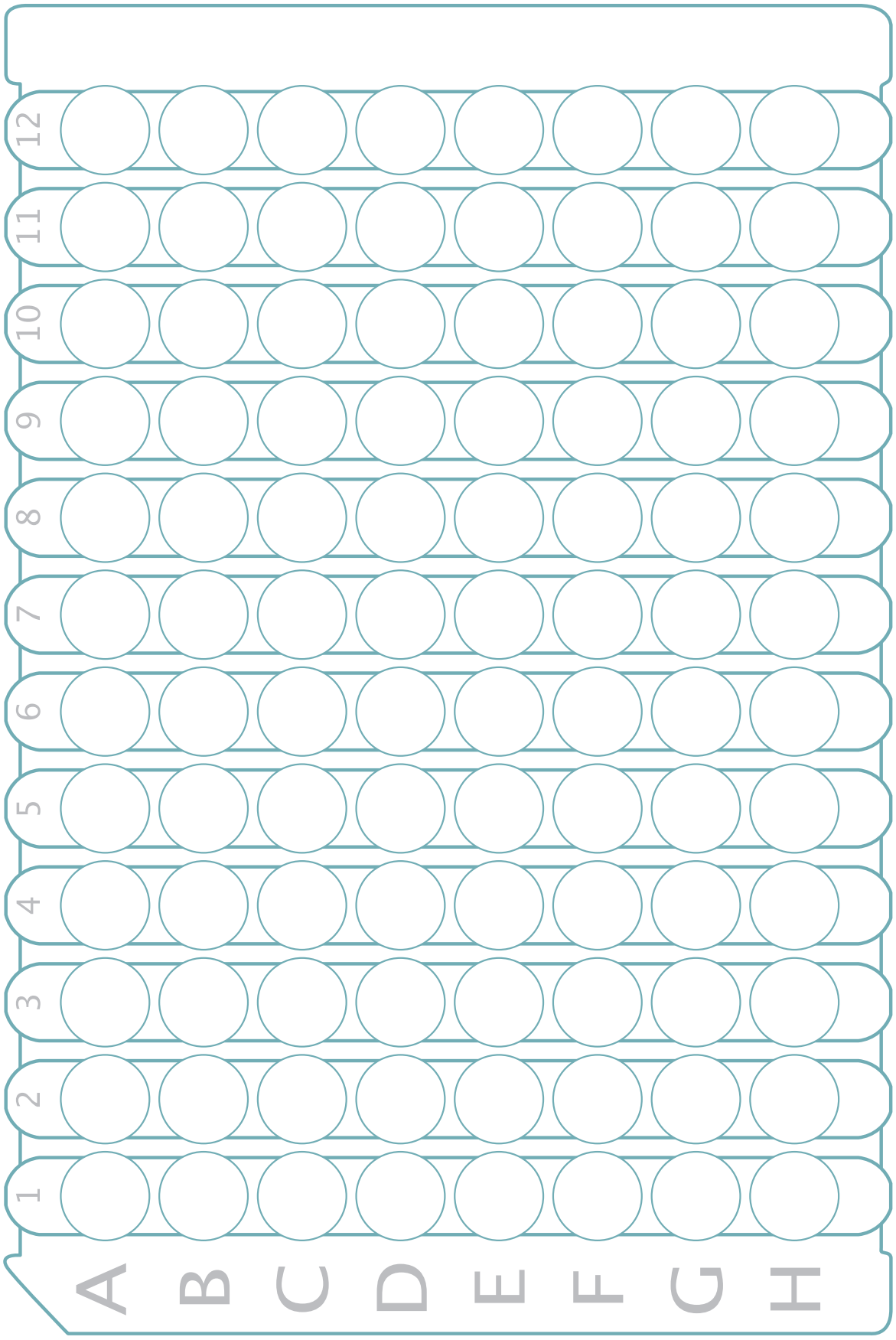
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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