Quantikine® ELISA

Mouse CCL11/Eotaxin Immunoassay

Catalog Number MME00

For the quantitative determination of mouse Eotaxin 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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614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
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19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
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24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
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INTRODUCTION

Mouse Eotaxin is a member of the CC chemokine family of inflammatory and immunoregulatory cytokines. Mouse Eotaxin cDNA encodes a 97 amino acid (aa) residue precursor protein with a 23 aa residue predicted signal peptide that is cleaved to generate the 74 aa residue mature protein (1-3). Among CC chemokine family members, Eotaxin is functionally and structurally most related to the MCP/Eotaxin proteins. At the amino acid sequence level, mouse Eotaxin is 63%, 37%, 49%, and 47% identical to human Eotaxin-1, human Eotaxin-2, mouse JE/MCP-1 and mouse MCP-3/MARC, respectively (1-3). Constitutive Eotaxin mRNA expression has been detected in multiple tissues, including adult thymus, lymph node, muscle, skin, lung, and intestinal tract (1, 20). During development, Eotaxin mRNA expression is also detected in fetal liver cells, yolk sac endothelium and the endocardium (19). Eotaxin mRNA has been shown to be markedly and rapidly up-regulated in airway epithelium and alveolar macrophages following allergen challenge (1, 6-9). In addition, inflammatory cytokines such as IL-1, TNF-α, and IFN-γ have also been found to induce Eotaxin mRNA accumulation in fibroblasts, endothelial and epithelial cells (1, 6-8). Additional cell types in which Eotaxin expression has been detected include smooth muscle cells, (3, 9), chondrocytes (9), and eosinophils (3, 7).

Mouse Eotaxin has been shown to be a potent and selective chemoattractant for mouse eosinophils during inflammation and allergic reactions (1, 5, 18). In embryonic development, Eotaxin has also been shown to be involved in the growth of myeloid cell progenitors and the differentiation of mast cells (19). The activities of mouse Eotaxin have been shown to be mediated by the mouse CC chemokine receptor CCR3, a G protein-coupled, seven transmembrane domains receptor (4, 10-12). Unlike human CCR3, mouse CCR3 can be activated by mouse MIP-1α, as well as by mouse Eotaxin (12). At the amino acid sequence level, mouse CCR3 shows approximately 68% identity with human CCR3 (4, 13). CCR3 expression has been detected in mouse leukocytes, spleen, and liver (4, 10-12). In humans, besides being expressed in eosinophils, basophils and dendritic cells, CCR3 has been shown to be selectively expressed in T helper 2 (Th2), but not T helper 1 (Th1), cells (14-17). If this observation holds true for the mouse system, CCR3 may be a good marker for distinguishing between Th1 and Th2 cell differentiation.

The Quantikine® Mouse CCL11/Eotaxin Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse Eotaxin in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse Eotaxin and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant factor accurately. Results obtained using natural mouse Eotaxin 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 Eotaxin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse Eotaxin has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any Eotaxin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse Eotaxin 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 Eotaxin 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 Eotaxin Microplate	890587	Two 96 well polystyrene microplates (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse Eotaxin.	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 Eotaxin Standard	890589	3 vials of recombinant mouse Eotaxin 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 within 8 hours of reconstitution.
Mouse Eotaxin Control	890590	3 vials of recombinant mouse Eotaxin in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse Eotaxin Conjugate	890588	23 mL of a polyclonal antibody specific for mouse Eotaxin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-21	895215	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-3	895436	2 vials (21 mL/vial) of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial) 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	8 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 or citrate 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 plasma has not been validated for use in this assay.*
Grossly hemolyzed or lipemic samples are not suitable for use in this assay.

SAMPLE PREPARATION

Serum and plasma samples require a 4-fold dilution. A suggested 4-fold dilution is 30 µL of sample + 90 µL of Calibrator Diluent RD5-3.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

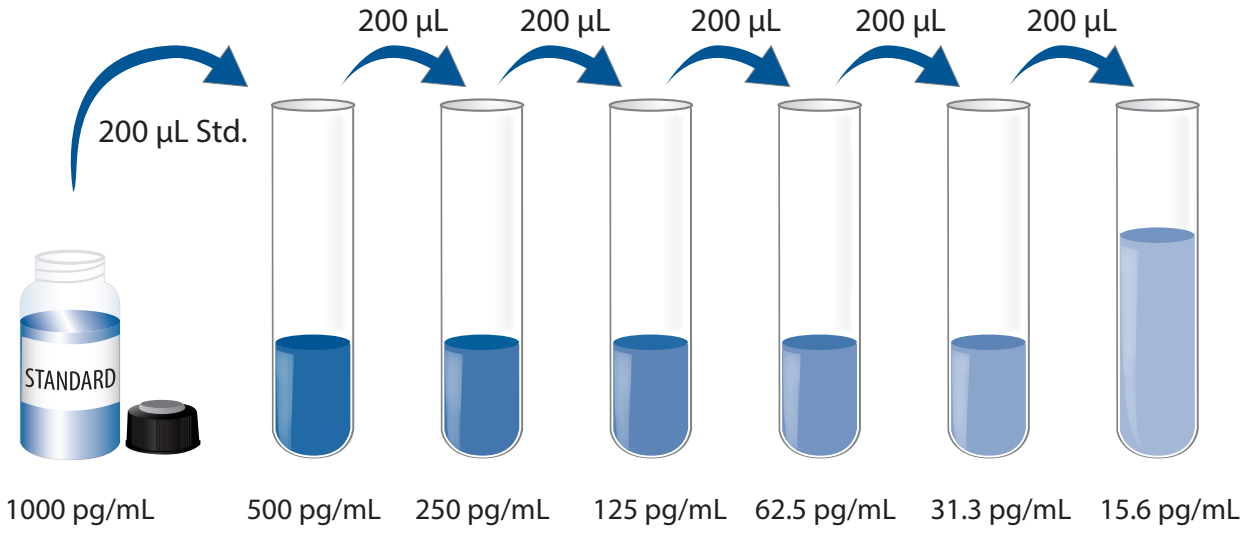
Mouse Eotaxin Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. 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 Eotaxin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse Eotaxin 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 dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse Eotaxin Standard (1000 pg/mL) serves as the high standard. 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 reagents, standard dilutions, control, and samples as directed by 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 RD1-21 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 samples and standards 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 auto washer. 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 Eotaxin 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

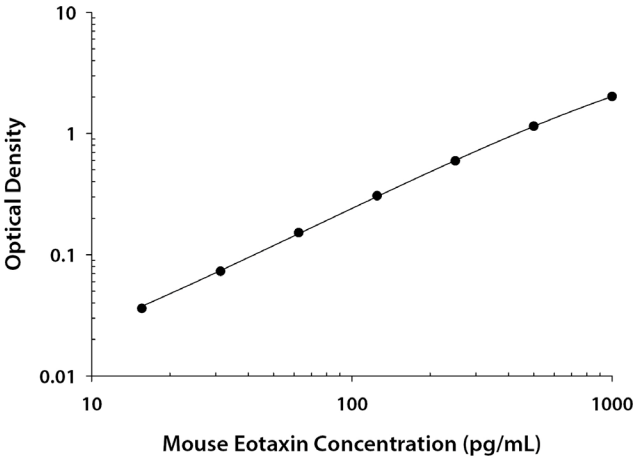
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 Eotaxin 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	0.014 0.015	0.014	—
15.6	0.049 0.051	0.050	0.036
31.3	0.087 0.087	0.087	0.073
62.5	0.164 0.167	0.166	0.152
125	0.323 0.318	0.320	0.306
250	0.609 0.606	0.608	0.594
500	1.168 1.156	1.162	1.148
1000	2.024 2.042	2.033	2.019

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)	49	197	466	46	179	446
Standard deviation	3.2	7.2	30	3.8	11.7	28.7
CV (%)	6.5	3.7	6.4	8.3	6.5	6.4

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates (n=6)	96	82-103%
Serum* (n=5)	98	82-111%
EDTA plasma* (n=5)	100	83-118%
Citrate plasma* (n=5)	97	81-114%

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

SENSITIVITY

The minimum detectable dose (MDD) of mouse Eotaxin is typically less than 3.0 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 Eotaxin 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 Eotaxin were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. 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	595	———	———
	1:2	284	298	95%
	1:4	136	149	91%
	1:8	70	74	95%
	1:16	33	37	89%
Serum*	Neat	399	———	———
	1:2	208	200	104%
	1:4	109	100	109%
	1:8	52	50	104%
	1:16	24	25	96%
EDTA plasma*	Neat	535	———	———
	1:2	289	268	108%
	1:4	142	134	106%
	1:8	71	67	106%
	1:16	32	34	94%
Citrate plasma*	Neat	527	———	———
	1:2	281	264	106%
	1:4	135	132	102%
	1:8	67	66	102%
	1:16	32	33	97%

*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 Eotaxin in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=20)	975	650-1592	316
EDTA plasma (n=20)	2268	1094-4132	722
Citrate plasma (n=20)	1146	466-2184	468

SPECIFICITY

This assay recognizes natural and recombinant mouse Eotaxin.

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

Recombinant mouse:

C10
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
JE/MCP-1
KC
Leptin
LIF
MARC/MCP-3
MCP-5
M-CSF
MIP-1α
MIP-1β
MIP-2
RANTES
SCF
TNF-α
Tpo
VEGF

Recombinant rat:

CINC-1

Recombinant human:

Eotaxin
GROα
GROβ
IL-8
IP-10
MIP-1α
MIP-1β
RANTES

Recombinant human Eotaxin cross-reacts approximately 0.062% in this assay.

Rat serum (diluted 4-fold into Calibrator Diluent RD5-3) was read as an unknown in this assay. The diluted rat serum measured 113 pg/mL of mouse Eotaxin equivalent. Upon further dilution, the dose-response curve of rat serum was parallel to the mouse Eotaxin standard curve. Rat serum samples must be diluted at least 4-fold prior to assay. Rat cell culture supernates may be assayed undiluted.

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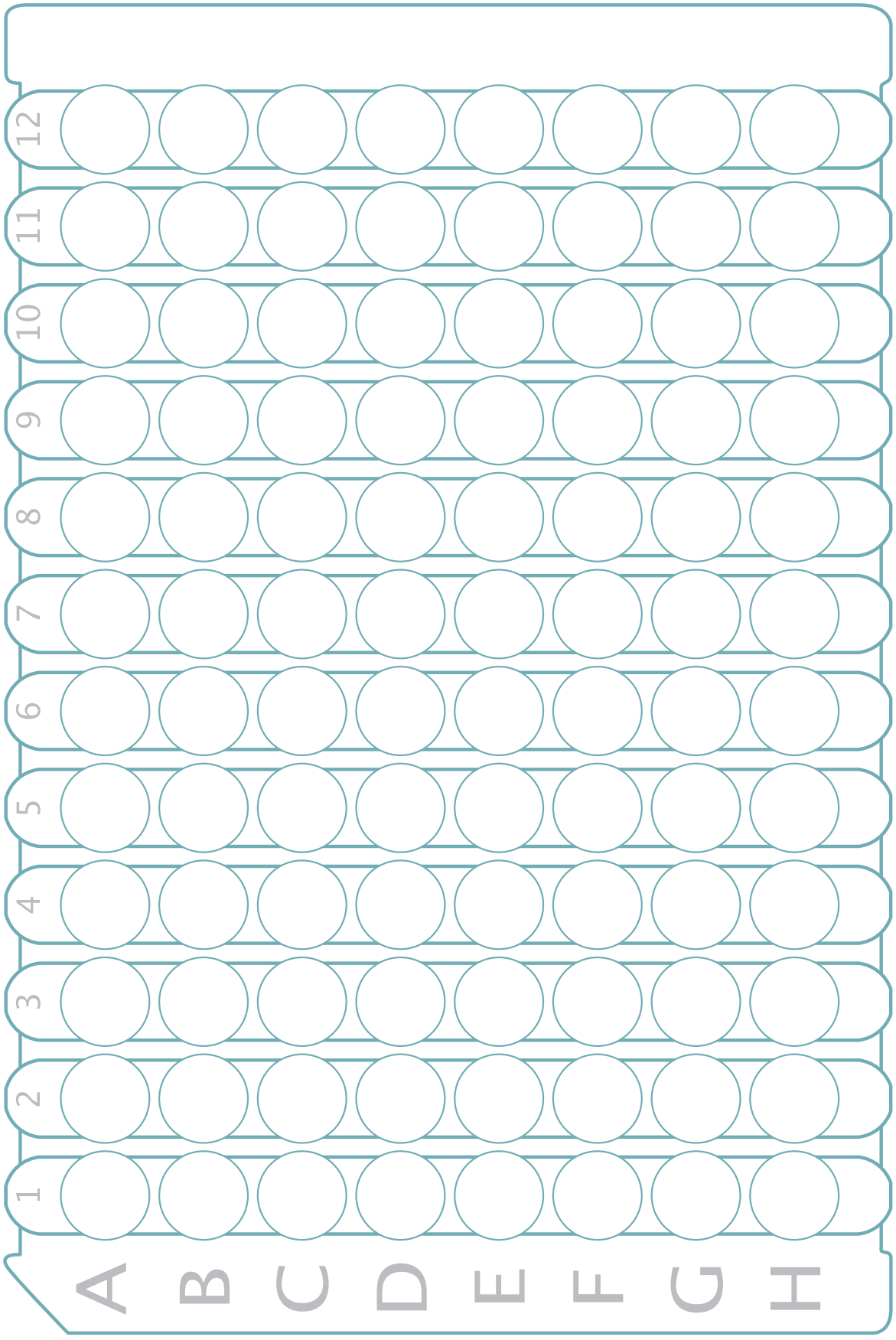
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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