Quantikine® ELISA

Mouse/Rat CCL5/RANTES Immunoassay

Catalog Number MMR00

For the quantitative determination of mouse/rat Regulated upon Activation, Normal T cell Expressed and presumably Secreted (RANTES) concentrations in cell culture supernates, serum, and platelet-poor plasma.

Note: The standard reconstitution method has changed. Please 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

RANTES (Regulated upon Activation, Normal T cell-expressed, and presumably Secreted), also known as CCL5, a member of the CC chemokine family of inflammatory and immunoregulatory cytokines, initially discovered by subtractive hybridization as a T cell-specific molecule (1, 2). Mouse RANTES cDNA encodes a 91 amino acid (aa) residue precursor protein with a presumed signal peptide of 23 aa residues that is cleaved to generate the 68 aa residue mature protein (3). At the amino acid sequence level, mouse RANTES is 84% identical to human RANTES. Both human and mouse RANTES exhibit cross species activity. Naturally occurring human RANTES has been found to be a mixture of the 68 aa residue mature protein and a 66 aa residue amino-terminally truncated form (4, 5). Cells known to express RANTES include keratinocytes, eosinophils, platelets, endothelium, fibroblasts, respiratory epithelium, astrocytes, vascular smooth muscle, and $CD4^+$ and $CD8^+$ T cells (6-14). As suggested by its acronym, RANTES production by the various cell types is induced in response to cytokine stimulation (8-13).

Like other CC chemokines, RANTES is a monocyte chemoattractant (2). RANTES can also chemoattract unstimulated $CD4^+/CD45RO^+$ memory T cells and stimulated $CD4^+$ and $CD8^+$ T cells with the naive and memory phenotypes. In addition, RANTES can chemoattract and degranulate eosinophils, as well as chemoattract and induce histamine release from basophils. Human RANTES has been shown to be an inhibitor of HIV infection of human mononuclear cells (15, 16). Several CC chemokine receptors, including CCR-1, CCR-3, CCR-4, and CCR-5, have been shown to bind RANTES and subsequently to transduce a signal by increasing the intracellular calcium ion level (2, 17-21).

The Quantikine® Mouse/Rat CCL5/RANTES Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse or rat RANTES levels in cell culture supernates, serum, and platelet-poor plasma. It contains *E. coli*-expressed recombinant mouse RANTES as well as antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate recombinant RANTES. Results obtained using natural mouse or rat RANTES 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 mouse and rat RANTES.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse/rat RANTES has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any RANTES present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse/rat RANTES 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 RANTES 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.
- It is recommended that the samples be pipetted within 15 minutes.
- 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.

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.

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/Rat RANTES Microplate	890609	Two 96 well polystyrene microplates (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse/rat RANTES.	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/Rat RANTES Standard	890611	Recombinant mouse RANTES 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 RANTES Control	890612	Recombinant mouse RANTES 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 RANTES Conjugate	890610	23 mL of a polyclonal antibody specific for mouse/rat RANTES conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-40	895513	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-16	895302	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.

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.

Platelet-poor Plasma - Collect plasma on ice using EDTA or citrate as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. An additional centrifugation step of the plasma at 10,000 x g for 10 minutes at 2-8 °C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Heparin plasma is not recommended for use in this assay.*
Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.

RANTES is present in platelet granules and is released upon platelet activation. Therefore, to measure circulating levels of RANTES, platelet-free plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical and Laboratory Standards Institute (CLSI), result in incomplete removal of platelets from blood. This will cause variable and irreproducible results for assays of factors released by platelet activation.

SAMPLE PREPARATION

Mouse serum and platelet-poor plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD5-16.

Rat serum samples require a 50-fold dilution. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5-16.

Rat platelet-poor plasma samples require a 5-fold dilution. A suggested 5-fold dilution is 30 µL of sample + 120 µL of Calibrator Diluent RD5-16.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

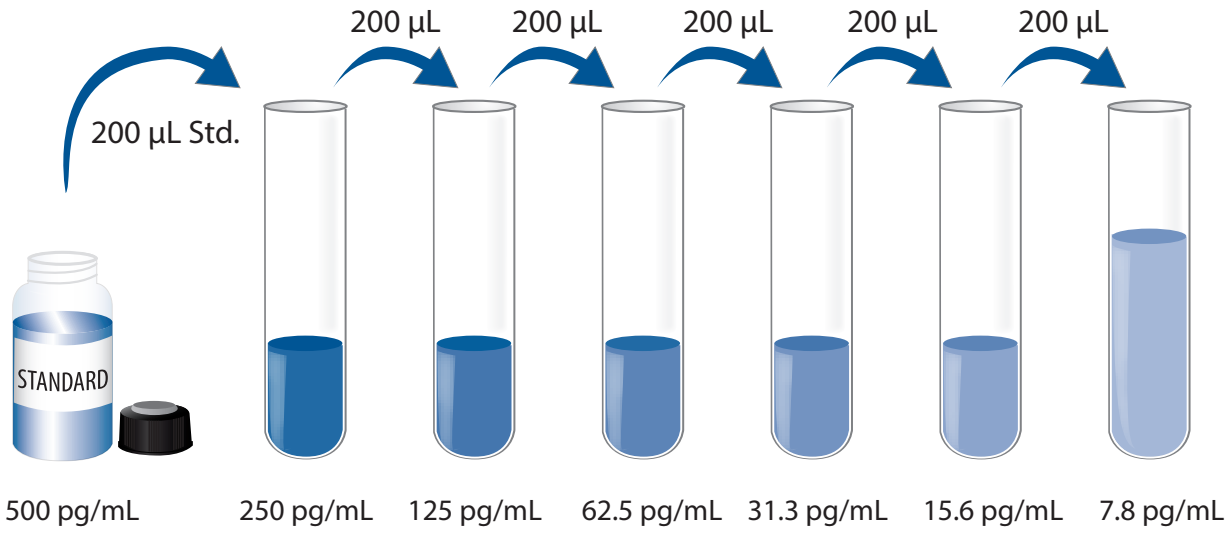
Mouse/Rat RANTES Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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/Rat RANTES Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse/Rat RANTES 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 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse/Rat RANTES 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 samples, control, and standards be assayed in duplicate.

1. Prepare reagents, standard curve dilutions, 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 RD1-40 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.

   Note: *Samples must be pipetted within 15 minutes.*
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 RANTES 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

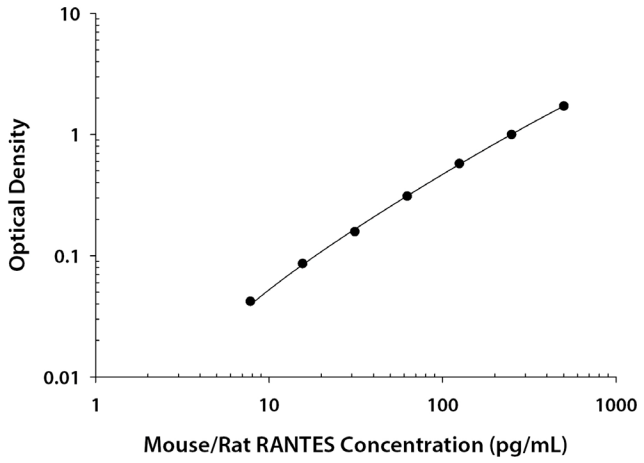
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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/rat RANTES 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	0.030 0.031	0.030	—
7.8	0.071 0.072	0.072	0.042
15.6	0.110 0.122	0.116	0.086
31.3	0.184 0.193	0.188	0.158
62.5	0.329 0.350	0.340	0.310
125	0.587 0.625	0.606	0.576
250	1.017 1.043	1.030	1.000
500	1.703 1.793	1.748	1.718

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)	18.8	104	198	20.1	109	208
Standard deviation	0.7	1.9	4.1	1.6	5.6	11.5
CV (%)	3.7	1.8	2.1	8.0	5.1	5.5

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates (n=8)	108	98-115%
Serum* (n=5)	97	90-107%
Platelet-poor EDTA plasma* (n=5)	99	86-110%
Platelet-poor citrate plasma* (n=5)	102	95-110%

*Samples were diluted prior to assay.

SENSITIVITY

The minimum detectable dose (MDD) of mouse/rat RANTES is typically less than 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 RANTES produced at R&D Systems®.

LINEARITY

To assess the linearity of the assay, four or more samples containing and/or spiked with various concentrations of mouse/rat RANTES in each matrix were diluted with calibrator diluent and then assayed.

Mouse Samples	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Cell culture supernates	Spiked	313	———	———
	1:2	153	156	98%
	1:4	71	78	91%
	1:8	34	39	87%
	1:16	17	20	85%
Serum*	Spiked	284	———	———
	1:2	145	142	102%
	1:4	72	71	101%
	1:8	37	36	103%
	1:16	19	18	106%
Platelet-poor EDTA plasma*	Spiked	284	———	———
	1:2	148	142	104%
	1:4	73	71	103%
	1:8	36	36	100%
	1:16	18	18	100%
Platelet-poor citrate plasma*	Spiked	264	———	———
	1:2	130	132	98%
	1:4	62	66	94%
	1:8	30	33	91%
	1:16	15	16	94%

Rat Samples	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Serum*	Neat	181	———	———
	1:2	93	91	103%
	1:4	47	45	104%
	1:8	23	23	103%
	1:16	11	11	98%
EDTA plasma*	Neat	162	———	———
	1:2	84	81	104%
	1:4	42	41	102%
	1:8	23	20	115%
	1:16	11	10	110%

*Samples were diluted prior to assay.

SAMPLE VALUES

Serum/Platelet-poor Plasma - Samples were evaluated for the presence of mouse and rat RANTES in this assay.

Mouse Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	94	70-116	17
EDTA plasma (n=10)	85	72-146	28
Citrate plasma (n=6)	63	52-74	8

Rat Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	7170	4400-18,750	4317
EDTA plasma	1214	780-1860	416

SPECIFICITY

This assay recognizes natural and recombinant mouse and rat RANTES.

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/rat RANTES control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C10
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
LIF
MARC
MCP-5
M-CSF
MIP-1α
MIP-1β
MIP-2
SCF
TNF-α
Tpo
VEGF

Recombinant rat:

CINC-1

Recombinant human:

GROβ
IP-10
IL-8
MIP-1α
MIP-1β

Recombinant human RANTES cross-reacts approximately 1.3% in this assay.

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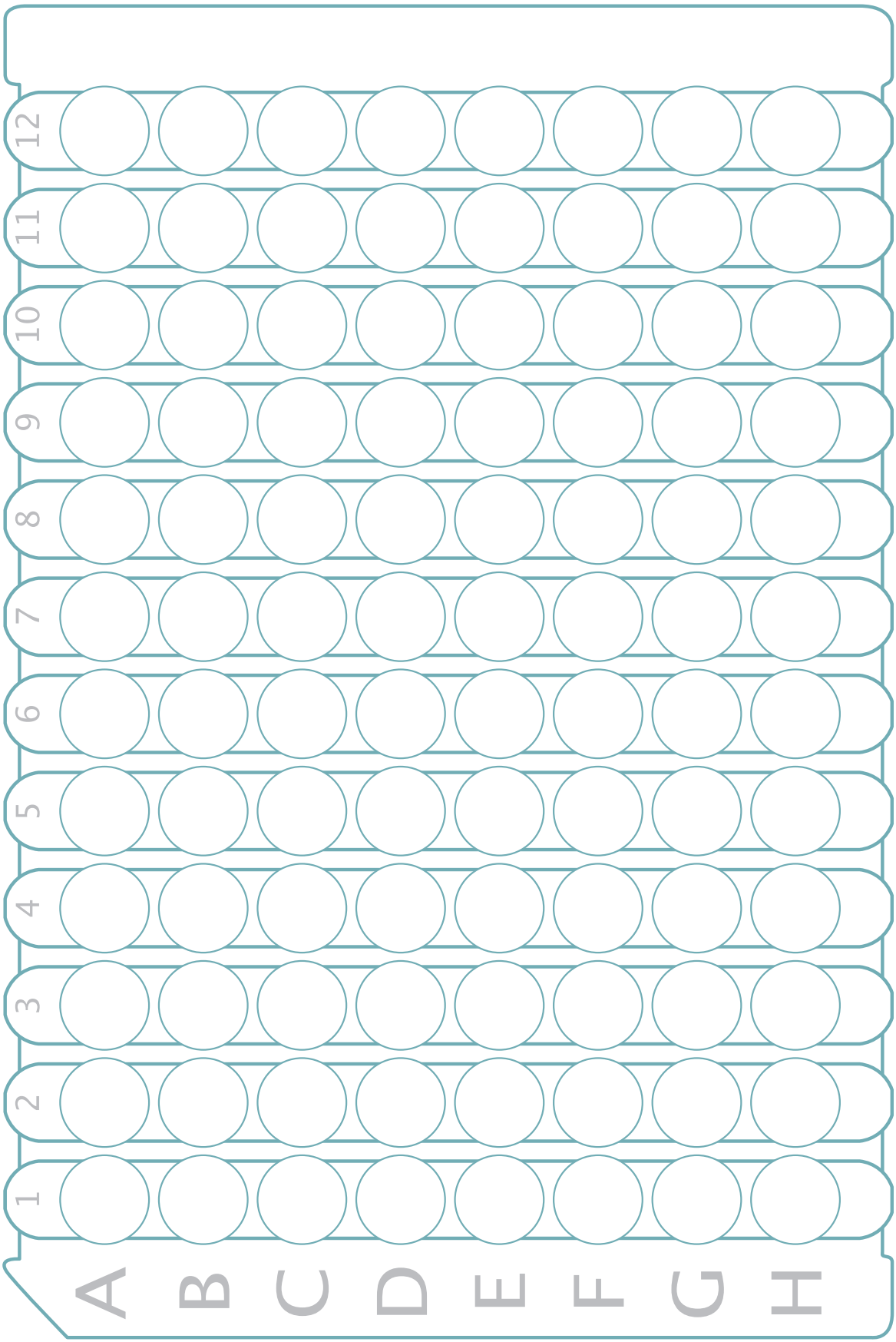
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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