Quantikine® ELISA

Mouse CCL17/TARC Immunoassay

Catalog Number MCC170

For the quantitative determination of mouse Thymus and Activation-Regulated Chemokine (TARC) 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

Thymus and Activation-Regulated Chemokine (TARC/CCL17) is a member of the CC or β-chemokine family (1, 2). Mouse TARC cDNA encodes a highly basic 93 amino acid (aa) residue precursor protein with a 23 aa residue putative signal peptide that is cleaved to generate the 70 aa residue secreted protein. The mature protein is not glycosylated and has a predicted molecular weight of 8.0 kDa (1). Mouse TARC shares 64% and 83% aa sequence identity with human and rat TARC, respectively (3, 4). Among other β-chemokine family members, TARC is most closely related to MDC, sharing approximately 33% aa sequence homology (5). TARC mRNA is constitutively expressed at high levels in thymic dendritic cells and at lower levels in lymph node dendritic cells in the lung, colon and small intestine (1, 5). Additional cell types that have been shown to express TARC include keratinocytes (6), monocytes (7), $CD4^+$ T cells (8), fibroblasts (9), bronchial epithelial cells (10), and Reed-Sternberg cells (11).

The chemokine receptor CCR4 has been shown to be a high-affinity functional receptor for TARC (12). Mouse and human CCR4 share 85% aa sequence identity (13, 14). In humans, CCR4 expression can be detected on Th2 $CD4^+$ T cells (12, 15), basophils (13), IL-2 activated NK cells (16), and platelets (17). Although CCR8 has also been reported to bind TARC, this finding is controversial (18, 19).

Recombinant TARC has been shown to chemoattract T cell lines and CCR4-transfected cells (5). Mouse TARC has no chemotactic activity on naive peripheral $CD4^+$ T cells but is a chemoattractant for memory/effector $CD4^+$ T cells with a preference for Th2 cells (20). One of the principal functions of TARC may be to recruit effector/memory T helper cells to antigen presenting dendritic cells at sites of inflammation (20). TARC has been shown to selectively recruit skin-homing CLA^+ memory $CD4^+$ T cells into skin during times of immune challenge (21). TARC has been shown to chemoattract IL-2 activated NK cell (16) and to induce platelet aggregation and degranulation (17).

The Quantikine Mouse TARC Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse TARC levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse TARC and antibodies raised against the recombinant factor. Results obtained for naturally occurring mouse TARC showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that this immunoassay can be used to determine relative mass values for natural mouse TARC.

PRINCIPLE OF THE ASSAY

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

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.

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 TARC Microplate	890933	96 well microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse TARC.	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 TARC Standard	890935	2.5 ng of recombinant mouse TARC in a buffered protein base, with preservatives; lyophilized.	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Mouse TARC Control	890936	1 vial of recombinant mouse TARC in a buffered protein base with preservatives; lyophilized. The concentration range of mouse TARC after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.	
Mouse TARC Conjugate	890934	12 mL of a polyclonal antibody against mouse TARC conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895038	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-12	895214	21 mL of diluted animal serum 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.

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 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 and citrate plasma have not been validated for use in this assay.*
Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

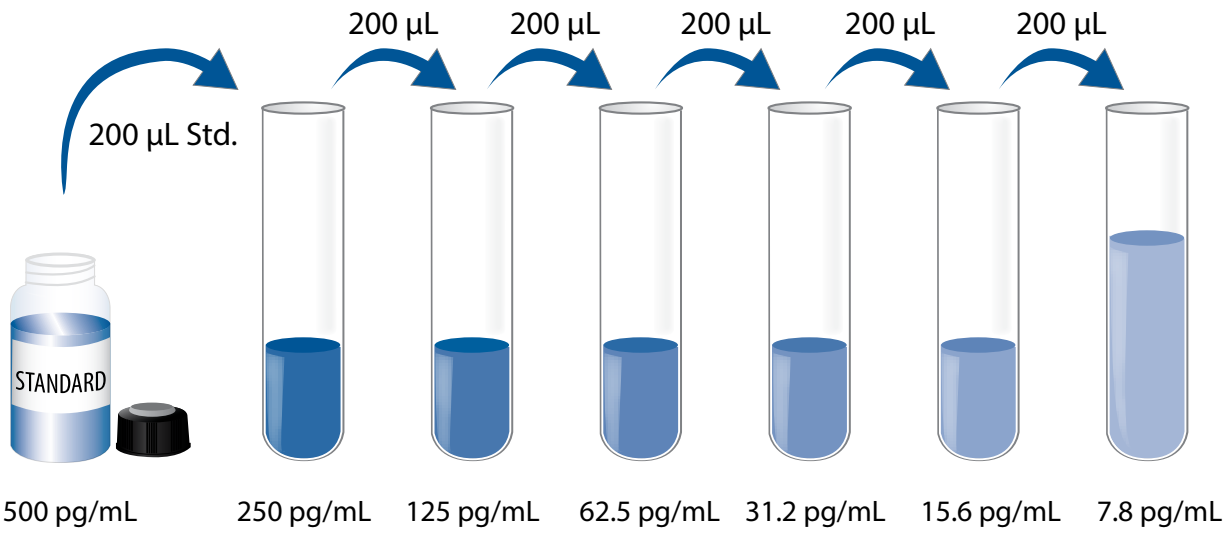
Mouse TARC Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

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 TARC Standard - Reconstitute the Mouse TARC Standard with 5.0 mL of Calibrator Diluent RD6-12. 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 test tubes. Pipette 200 µL of Calibrator Diluent RD6-12 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted mouse TARC Standard serves as the high standard (500 pg/mL). Calibrator Diluent RD6-12 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 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 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.

CALCULATION OF RESULTS

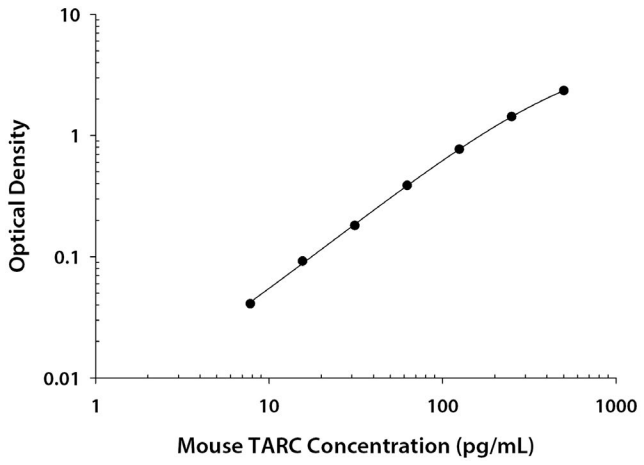
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 TARC 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	0.051 0.054	0.052	—
7.8	0.094 0.092	0.093	0.041
15.6	0.144 0.145	0.144	0.092
31.2	0.235 0.231	0.233	0.181
62.5	0.435 0.445	0.440	0.388
125	0.807 0.839	0.823	0.771
250	1.458 1.504	1.481	1.429
500	2.271 2.527	2.399	2.347

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)	27	89	278	29	98	310
Standard deviation	1.3	2.3	7.8	2.2	6.9	17.3
CV (%)	4.8	2.6	2.8	7.6	7.0	5.6

RECOVERY

The recovery of mouse TARC 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)	94	85-113%
Serum (n=6)	94	82-103%
EDTA plasma (n=6)	94	83-109%

SENSITIVITY

The minimum detectable dose (MDD) of mouse TARC is typically less than 5 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 highly purified *E. coli*-expressed recombinant mouse TARC 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 TARC in each matrix were diluted with Calibrator Diluent and assayed. 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	311	———	———
	1:2	145	156	93%
	1:4	75	78	96%
	1:8	38	39	97%
	1:16	19	19	100%
Serum	Spiked	367	———	———
	1:2	190	183	104%
	1:4	92	92	100%
	1:8	53	46	115%
	1:16	23	23	100%
EDTA plasma	Spiked	382	———	———
	1:2	202	191	106%
	1:4	106	96	110%
	1:8	49	48	102%
	1:16	25	24	104%

SAMPLE VALUES

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

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=20)	70	20-123	30
EDTA plasma (n=20)	69	32-100	20

Cell Culture Supernates:

Mouse splenocytes (1 x 10^6 cells/mL) were cultured for 3 days in RPMI plus 10% fetal calf serum supplemented with 50 µM β-mercaptoethanol and 10 ng/mL recombinant human IL-2. An aliquot of the cell culture supernate was removed, assayed for mouse TARC, and measured 246 pg/mL.

Mouse lung conditioned media (2 lungs, 1-2 mm pieces in 40 mL of medium) were collected after culturing for 6 days in RPMI with 10% fetal calf serum. An aliquot of the cell culture supernate was removed, assayed for mouse TARC, and measured 421 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse TARC.

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

Recombinant mouse:

C10
Eotaxin
Flt-3 Ligand
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/IL-23 p40
IL-12 p70
IL-13
IL-17
IL-18
JE/MCP-1
KC
LIF
MARC
MCP-5
M-CSF
MIP-1α
MIP-2
OPG
OSM
PlGF-2
RANTES
SCF
TNF-α
TNF RI
TNF RII
Tpo
TRANCE
VEGF
VEGF R1

Recombinant human TARC cross-reacts approximately 0.1% in this assay.

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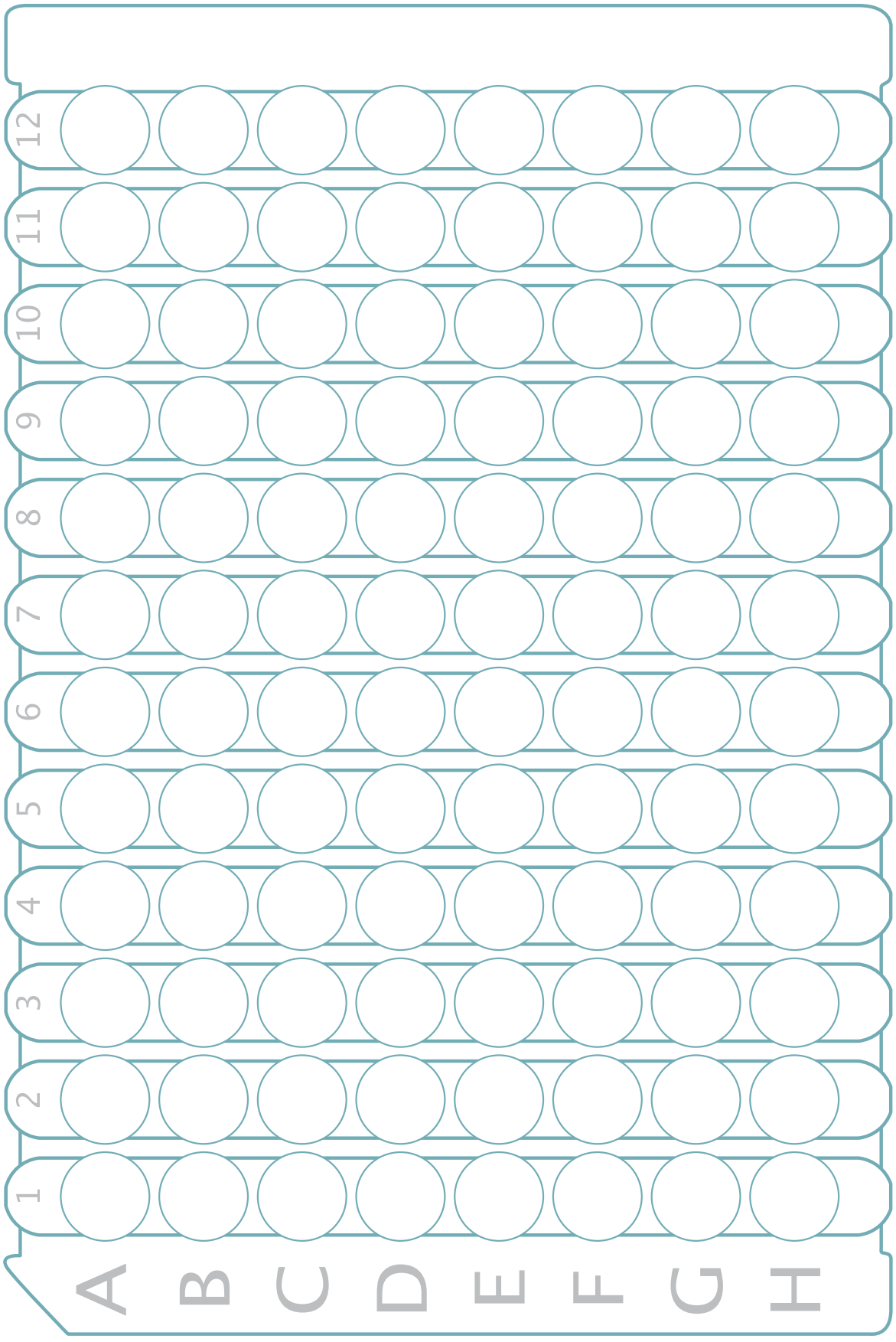
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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