Quantikine® ELISA

Mouse TRANCE/RANK L/TNFSF11 Immunoassay

Catalog Number MTR00

For the quantitative determination of mouse TNF-Related Activin-induced Cytokine (TRANCE) concentrations in cell culture supernates and serum.

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

Mouse TRANCE (also known as TNFSF11, RANK L, OPG-Ligand and ODF) is a member of the TNF superfamily that appears to play a central role in both immune response and bone morphogenesis (1, 2). Like other members of the TNF superfamily, the presence of TRANCE seems to be crucial both during development and adult life. In the immune system of the fetus, there appears to be an absolute requirement for TRANCE in lymph node development, and a contribution from TRANCE during Peyer's patch formation. In addition, TRANCE drives both B and T cell maturation at early maturation stages. In the adult, TRANCE impacts T cell-dendritic cell (DC) interaction. Following TCR engagement, TRANCE is upregulated on T cells and subsequently binds to dendritic cell RANK/TNFRSF11A, promoting both DC survival and interleukin production (1-5). During bone development and remodeling, TRANCE expression by osteoblasts in conjunction with M-CSF induces osteoclast formation and activation, resulting in bone resorption. This allows for subsequent bone deposition and remodeling (1, 6, 7). TRANCE also plays a role in pathological osteoporosis. Its inappropriate expression by activated lymphocytes and osteoblasts results in excessive joint and bone resorption vs. deposition (1, 8-11).

Mouse TRANCE is a 45 kDa, 316 amino acid (aa) type II transmembrane glycoprotein that exists naturally as a nondisulfide-linked homotrimer (1, 11-15). The molecule has a 47 aa cytoplasmic domain, a 23 aa transmembrane segment, and a 246 aa extracellular region (14). Within its extracellular region, it contains a 159 aa TNF domain. The 177 aa soluble TRANCE is generated by TACE metalloproteinase cleavage of membrane-bound TRANCE (12, 16). Although both membrane and soluble TRANCE are bioactive, the homeostatic form of TRANCE may be the membrane-bound form (9, 17), while soluble TRANCE may signal underlying pathology (9). Cells known to express TRANCE include odontoblasts and ameloblasts (18), osteoblasts (13, 19, 20), $CD4^+$ and $CD8^+$ T cells (4), fetal and adult chrondrocytes (20), megakaryocytes (20), bronchial and intestinal epithelium (20), fetal and adult neurons (20), fibroblasts (20), and skeletal muscle cells (20). Mouse TRANCE is active on human cells (12). It shows 85% and 96% aa sequence identity to human and rat TRANCE, respectively (12, 15, 21).

TRANCE binds and signals via a membrane-bound TNF receptor superfamily member named RANK/TNFRSF11A (15). TRANCE also binds a naturally occurring 55 kDa soluble receptor antagonist named osteoprotegerin (or *protector of the bone*, also known as OPG/TNFSFR11B) (22). While RANK/TNFRSF11A mediates the osteoclastic activity associated with TRANCE, OPG, as both a monomer and disulfide-linked homodimer, binds both membrane and soluble TRANCE, abrogating the osteoclast effects associated with TRANCE (10, 13, 22, 23).

The Quantikine® Mouse TRANCE/RANK L/TNFSF11 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse TRANCE in cell culture supernates and serum. It contains NS0-expressed recombinant mouse TRANCE and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate recombinant mouse TRANCE accurately. Results obtained using natural mouse TRANCE 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 TRANCE.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An affinity purified polyclonal antibody specific for mouse TRANCE has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any TRANCE present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse TRANCE 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 TRANCE 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.
- For best results, pipette reagents and samples into the center of each well.
- 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.

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 TRANCE/RANK L Microplate	890874	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse TRANCE.	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 TRANCE/RANK L Standard	890876	Recombinant mouse TRANCE 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 in a manual defrost freezer.*
Mouse TRANCE/RANK L Control	890221	Recombinant mouse TRANCE in a buffered protein base with preservatives; lyophilized. The assayed value of the control should be within the range specified on the label.	
Mouse TRANCE/RANK L Conjugate	890877	12 mL of a polyclonal antibody specific for mouse TRANCE 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 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.

Note: *Grossly hemolyzed or lipemic samples are not suitable for use in this assay.*

SAMPLE PREPARATION

Serum samples require a 2-fold dilution prior to assay. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD6-12.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

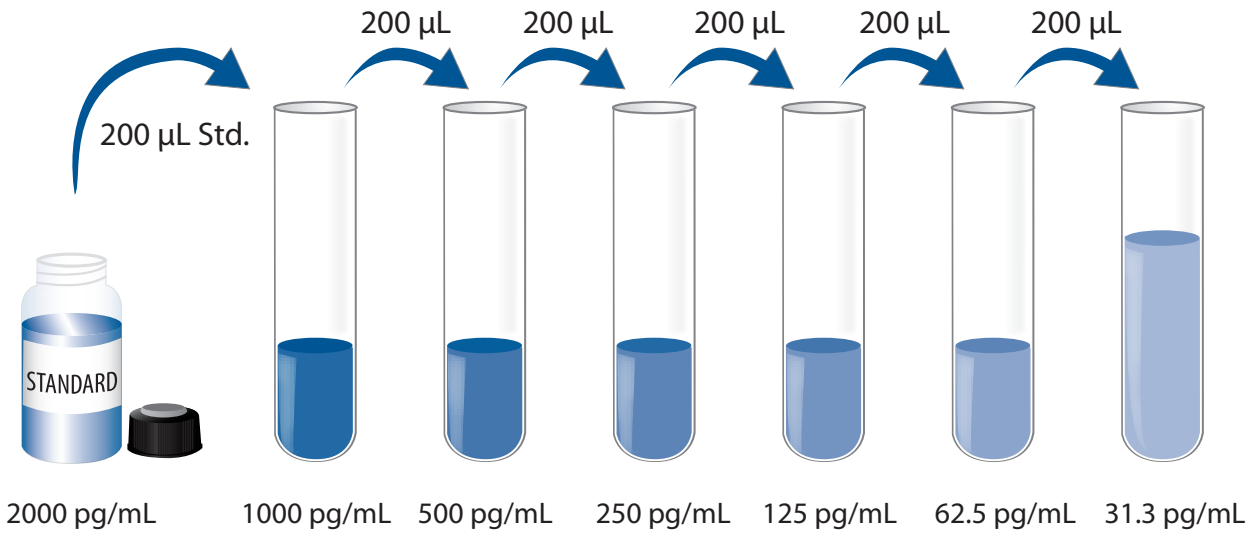
Mouse TRANCE/RANK L Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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 TRANCE/RANK L Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse TRANCE/RANK L Standard with Calibrator Diluent RD6-12. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 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 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 TRANCE/RANK L Standard (2000 pg/mL) serves as the high standard. 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 Mouse TRANCE/RANK L 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

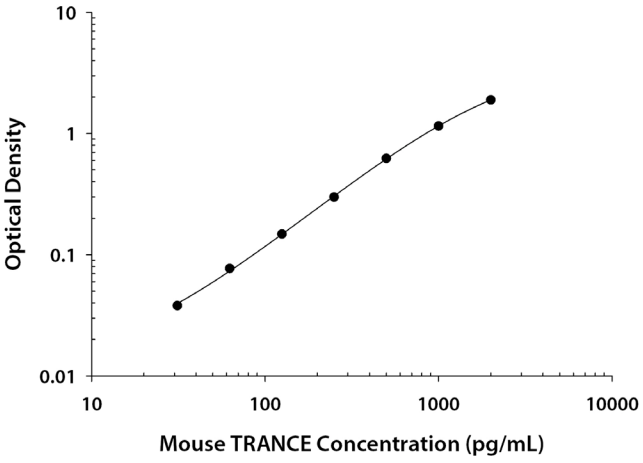
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 TRANCE 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	0.034 0.035	0.034	—
31.3	0.070 0.074	0.072	0.038
62.5	0.110 0.112	0.111	0.077
125	0.178 0.185	0.182	0.148
250	0.330 0.336	0.333	0.299
500	0.653 0.661	0.657	0.623
1000	1.183 1.190	1.186	1.152
2000	1.922 1.935	1.928	1.894

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)	99	261	830	111	285	886
Standard deviation	8.0	6.8	18.3	8.8	17.8	57.8
CV (%)	8.1	2.6	2.2	7.9	6.2	6.5

RECOVERY

The recovery of mouse TRANCE 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)	99	87-116%
Serum* (n=6)	93	80-104%

*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 mouse TRANCE were diluted with calibrator diluent and assayed.

Samples	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}}$ x 100
Cell culture supernate	Neat	961	———	———
	1:2	454	480	94%
	1:4	230	240	96%
	1:8	117	120	98%
	1:16	60	60	100%
Serum*	Spiked	699	———	———
	1:2	319	350	91%
	1:4	158	175	90%
	1:8	78	87	90%
	1:16	43	44	98%

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

SENSITIVITY

The minimum detectable dose (MDD) of mouse TRANCE is typically less than 5.0 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 highly purified NS0-expressed recombinant mouse TRANCE produced at R&D Systems®.

SAMPLE VALUES

Serum - Samples were evaluated for the presence of mouse TRANCE in this assay.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=40)	93	98	ND-195

ND=Non-detectable

Cell Culture Supernates - Mouse splenocytes (1 x 10^6 cells/mL) were cultured for 3 days in RPMI + 10% fetal bovine 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 TRANCE, and measured 439 pg/mL.

SPECIFICITY

This assay recognizes free natural and recombinant mouse TRANCE.

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

Recombinant mouse:

C10
CD40
Eotaxin
Fas Ligand
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 R
JE/MCP-1
KC
Leptin
LIF
MARC
MCP-5
M-CSF
MIP-1α
MIP-1β
MIP-2
OSM
PlGF-2
RANTES
SCF
TARC
TNF-α
TNF RI
TNF RII
Tpo
VEGF
VEGF R1/Flt-1

Recombinant human:

APRIL
DcR3
DR6
Fas
LIGHT
TRAIL

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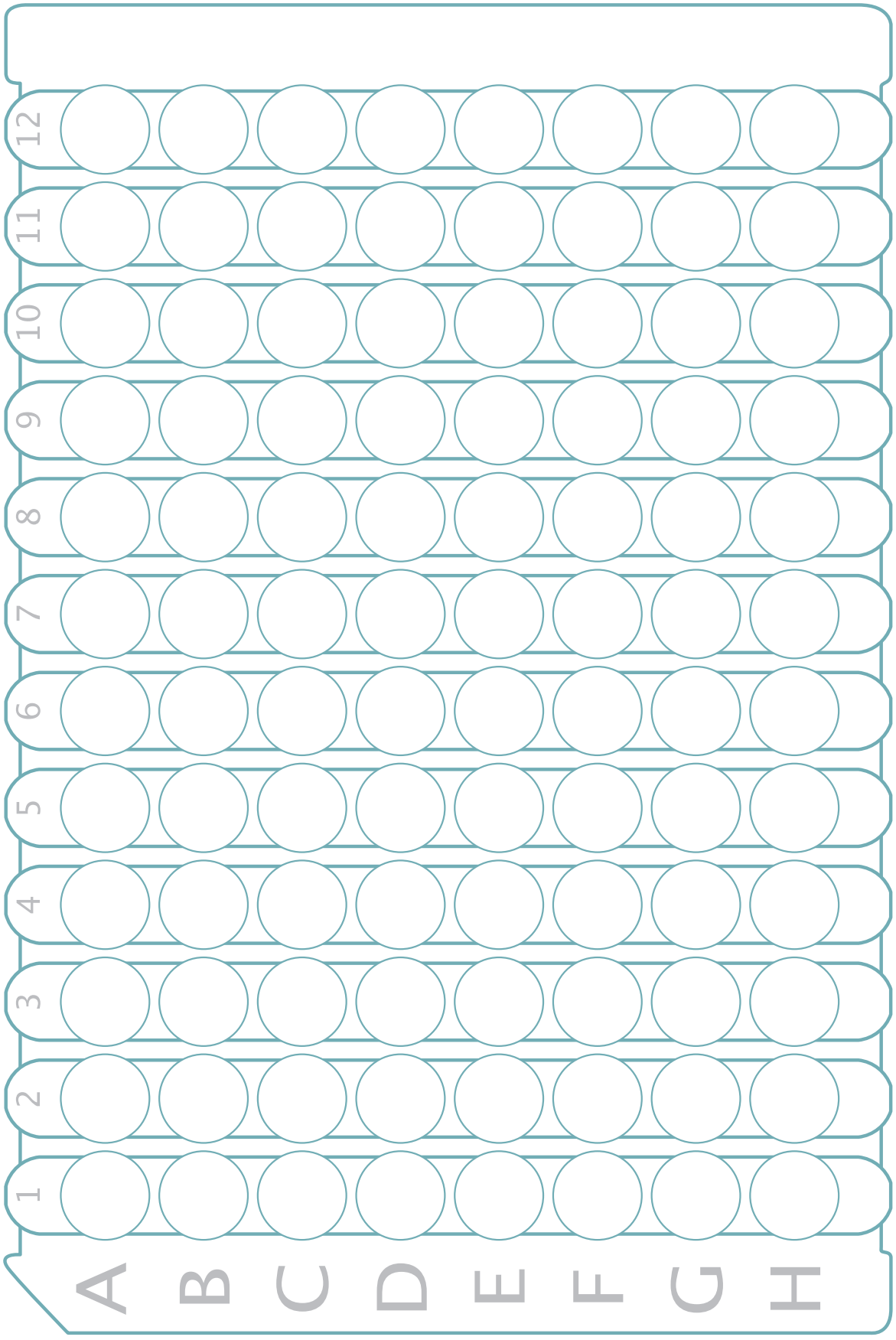
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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