Quantikine® ELISA

Mouse IL-1ra/IL-1F3 Immunoassay

Catalog Number MRA00

For the quantitative determination of mouse Interleukin 1 Receptor Antagonist (IL-1ra) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. 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.

TABLE OF CONTENTS

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INTRODUCTION

Mouse Interleukin-1 receptor antagonist (IL-1ra) is a 22-25 kDa glycoprotein produced by a variety of cell types that antagonizes IL-1 activity (1-3). It is a member of the IL-1 family of proteins that includes IL-1α and IL-1β. Although there is little amino acid (aa) identity (< 30%) among the three IL-1 family members, all molecules bind to the same receptors, all show a β-trefoil structure, all are physically placed on mouse chromosome # 2, and all are believed to have evolved from a common ancestral gene (1-4). Mouse IL-1ra is synthesized as a 178 aa precursor that contains a 26 aa signal sequence plus a 152 aa mature region. There is one intrachain disulfide bond and one potential N-linked glycosylation site (3, 5, 6). Mature mouse IL-1ra shares 90%, 77%, 80%, 80%, and 78% aa sequence identity to mature rat (4), human (7), porcine (8), canine (9), and equine (10) IL-1ra, respectively. In humans, three non-secreted IL-1ra isoforms have also been identified (11-14). These result from the use of alternate start sites or exon splicing. In mice, only one of the three human intracellular isoforms has been isolated. This mouse molecule is the ortholog of the 159 aa human intracellular isoform # 1 (6, 12). The mouse intracellular form differs from the secreted precursor by only three amino acids. Cells known to secrete IL-1ra include dermal fibroblasts (15), vascular smooth muscle cells (16), intestinal columnar epithelium (17), chondrocytes (18), macrophages (19), non-keratinized oral stratified squamous epithelium (20), mast cells (21), neutrophils and monocytes (22), Sertoli cells (23), and hepatocytes (24).

There are two type I transmembrane glycoprotein receptors for IL-1ra: the bioactive 80 kDa type I IL-1 receptor (IL-1 RI), and the inert (decoy) 65 kDa type II IL-1 receptor (IL-1 RII). IL-1ra binding to IL-1 RI competitively blocks IL-1 (α or β) binding to the same receptor. Unlike the IL-1/IL-1 RI complex, the IL-1ra/IL-1 RI complex cannot recruit the IL-1 receptor accessory protein that is required for signal transduction. This results in receptor ligation without cell activation (1, 25). IL-1ra also competitively blocks IL-1 binding to the decoy IL-1 RII. In this case, IL-1ra may actually potentiate IL-1 activity by cancelling the functions of both antagonists.

All activities attributed to IL-1ra are explained by its role as a competitive inhibitor of IL-1 binding to IL-1 RI (1, 2, 26, 27). In general, the ratio of IL-1ra to IL-1β is close to 1 in both health and disease (26, 28). To achieve total abrogation of IL-1 activity, more than 95% of all IL-1 RI receptors apparently need to be occupied by IL-1ra (29). Thus, local concentrations of secreted IL-1ra may be the critical determinants of IL-1 activity. The function of intracellular IL-1ra is less clear. While it would seem to be a simple competitor of IL-1 activity, its role may be limited to that of downmodulating non-specific inflammation associated with cell debris and death (14, 27). It has no action intracellularly; only when released through cell death would it then become a functional IL-1 antagonist.

The Quantikine® Mouse IL-1ra/IL-1F3 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse IL-1ra in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse IL-1ra and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse IL-1ra 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 IL-1ra.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse IL-1ra has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-1ra present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-1ra 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 IL-1ra 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.
- It is recommended that the samples be pipetted within 10 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 IL-1ra Microplate	890859	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse IL-1ra.	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 IL-1ra Standard	890861	2 vials of recombinant mouse IL-1ra in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a fresh standard and control for each assay. Discard after use.
Mouse IL-1ra Control	890144	2 vials of recombinant mouse IL-1ra in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse IL-1ra Conjugate	890860	12 mL of a polyclonal antibody specific for mouse IL-1ra 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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards.

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

REAGENT PREPARATION

Bring all reagents to room temperature before use.

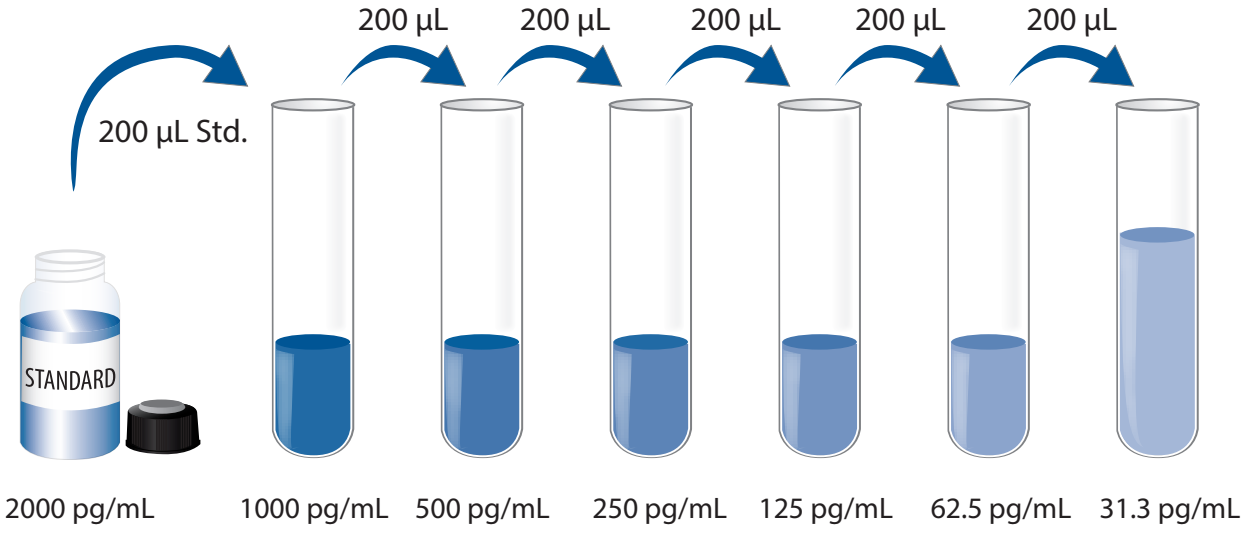
Mouse IL-1ra Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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 IL-1ra Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-1ra 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.

Pipette 200 µL of Calibrator Diluent RD6-12 into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse IL-1ra 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 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 RD1W to each well.
4. Add 50 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
   Note: *Samples must be pipetted within 10 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 IL-1ra Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
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 **on the benchtop. 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

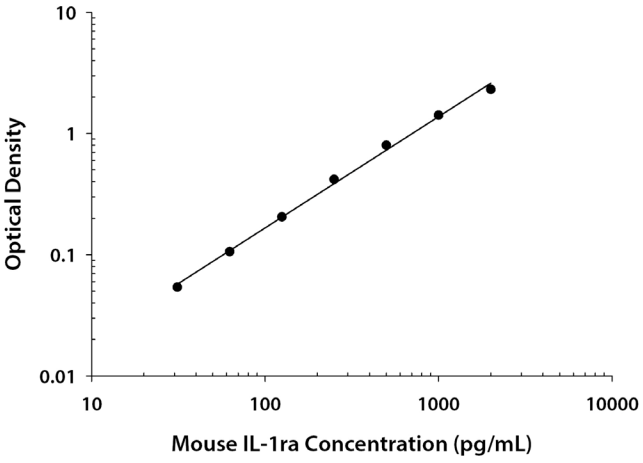
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the mouse IL-1ra concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the 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	0.073 0.075	0.074	—
31.3	0.124 0.133	0.128	0.054
62.5	0.175 0.185	0.180	0.106
125	0.276 0.282	0.279	0.205
250	0.490 0.493	0.492	0.418
500	0.859 0.888	0.874	0.800
1000	1.479 1.503	1.491	1.417
2000	2.382 2.391	2.386	2.312

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 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	26	26	27
Mean (pg/mL)	63	227	1226	66	225	1233
Standard deviation	5.1	12.2	47.2	6.1	12.5	29.9
CV (%)	8.1	5.4	3.8	9.2	5.6	2.4

RECOVERY

The recovery of mouse IL-1ra spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=5)	110	95-120%
Serum (n=4)	95	88-104%
EDTA plasma (n=4)	92	82-101%
Heparin plasma (n=4)	91	83-101%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse IL-1ra were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=7)	Serum (n=5)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	99	108	106	104
	Range (%)	89-111	106-112	103-110	102-106
1:4	Average % of Expected	94	110	100	102
	Range (%)	80-106	108-113	94-105	101-104
1:8	Average % of Expected	102	107	99	100
	Range (%)	99-107	102-111	93-104	98-103
1:16	Average % of Expected	103	102	97	95
	Range (%)	100-109	91-119	96-98	93-98

SENSITIVITY

Sixteen assays were evaluated and the minimum detectable dose (MDD) of mouse IL-1ra ranged from 4-13 pg/mL. The mean MDD was 7 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 IL-1ra produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse IL-1ra in this assay.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=20)	442	95	ND-2476
EDTA plasma (n=20)	94	65	ND-639
Heparin plasma (n=20)	266	100	66-1241

ND=Non-detectable

Cell Culture Supernates:

J774.A1 mouse reticulum cell sarcoma macrophage cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 10% fetal bovine serum, 100 ng/mL recombinant mouse IFN-γ, and 1 µg/mL LPS for 3 days. An aliquot of the cell culture supernate was removed, assayed for mouse IL-1ra, and measured 17.2 ng/mL.

Mouse lungs (1-2 mm pieces in 50 mL of medium) were cultured for 5 days in RPMI supplemented with 10% fetal bovine serum and stimulated with 50 ng/mL ConA. An aliquot of the cell culture supernate was removed, assayed for mouse IL-1ra, and measured 1057 pg/mL.

Mouse spleen cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum for 4 days. An aliquot of the cell culture supernate was removed, assayed for mouse IL-1ra, and measured 304 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-1ra.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the same factors at 50 ng/mL in a mid-range mouse IL-1ra control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

Eotaxin
Flt-3 Ligand
Follistatin
G-CSF
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-1 RII
IL-1 R4
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-10 R
IL-11
IL-12 p70
IL-13
IL-17
IL-18
JE/MCP-1
L-Selectin
MIG
OPG
TARC
TGF-β RII
Tie-2
TNF-α
TNF-β
TNF RI
Tpo
VEGF R2

Recombinant rat:

IL-1α
IL-1β
IL-1 R6

Recombinant cotton rat:

IL-1α
IL-1β

Recombinant porcine:

IL-1α
IL-1β

Recombinant human:

IL-1α
IL-1β
IL-1 RII
IL-1 R3
IL-1 R4
IL-1 R6
IL-1 R9

Recombinant rat IL-1ra cross-reacts approximately 12% in this assay.

Recombinant porcine IL-1ra and recombinant human IL-1ra cross-react approximately 0.4% in this assay.

Recombinant mouse IL-1 RI and recombinant human IL-1 RI interfere at concentrations > 2 ng/mL in this assay.

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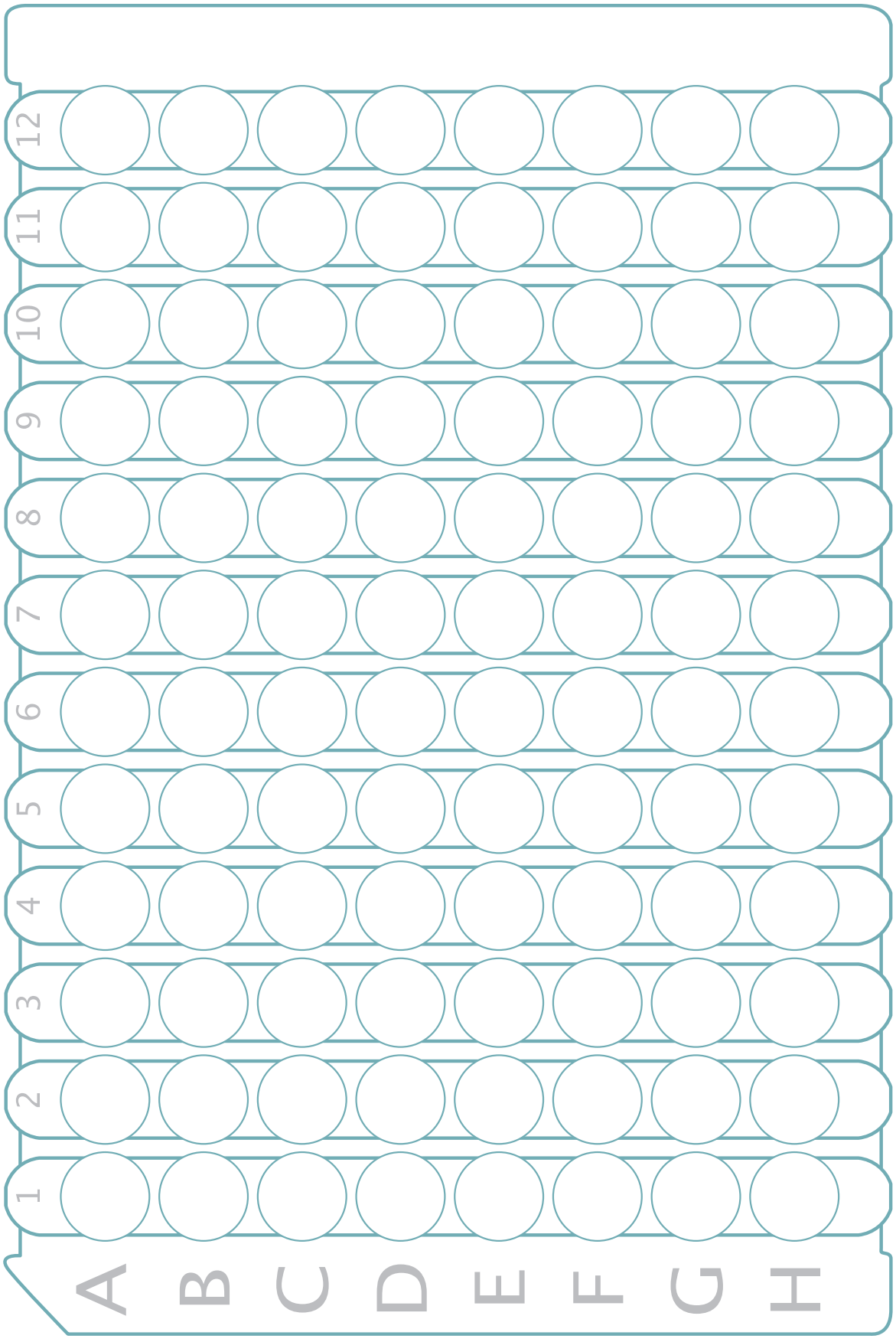
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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