Quantikine® ELISA

Rat IL-1β/IL-1F2 Immunoassay

Catalog Number RLB00
SRLB00
PRLB00

For the quantitative determination of rat Interleukin 1 beta (IL-1β) concentrations in cell culture supernates, serum, and plasma.

Note: Beginning March 2014, 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.

TABLE OF CONTENTS

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INTRODUCTION

The Interleukin 1 (IL-1) family of proteins consists of the classic members IL-1α, IL-1β, and IL-1ra, plus IL-18, IL-33, and IL-1F5-10. IL-1α and IL-1β bind to the same cell surface receptors and share biological functions (1). IL-1 is not produced by unstimulated cells of healthy mice with the exception of skin keratinocytes, some epithelial cells, and certain cells of the central nervous system. In response to inflammatory agents, infections, or microbial endotoxins, however, a dramatic increase in the production of IL-1 by macrophages and various other cell types is observed. IL-1β plays a central role in immune and inflammatory responses, bone remodeling, fever, carbohydrate metabolism, and GH/IGF-I physiology. Inappropriate or prolonged production of IL-1 has been implicated in a variety of pathological conditions including sepsis, rheumatoid arthritis, inflammatory bowel disease, acute and chronic myelogenous leukemia, insulin-dependent diabetes mellitus, atherosclerosis, neuronal injury, and aging-related diseases (2-5).

IL-1α and IL-1β are structurally related polypeptides that show approximately 25% homology at the amino acid level. Both are synthesized as 31 kDa precursors that are subsequently cleaved into mature proteins of approximately 17.5 kDa (6, 7). Cleavage of the IL-1β precursor by Caspase-1/ICE is a key step in the inflammatory response (2, 8). Neither IL-1α nor IL-1β contains a typical hydrophobic signal peptide (9-11), but evidence suggests that these factors can be secreted by non-classical pathways (12, 13). A portion of unprocessed IL-1α can be presented on the cell membrane and may retain biological activity (14). The precursor form of IL-1β, unlike the IL-1α precursor, shows little or no biological activity in comparison to the processed form (13, 15). Both unprocessed and mature forms of IL-1β are exported from the cell.

IL-1α and IL-1β exert their effects through immunoglobulin superfamily receptors that additionally bind IL-1ra. The 80 kDa transmembrane type I receptor (IL-1 RI) is expressed on T cells, fibroblasts, keratinocytes, endothelial cells, synovial lining cells, chondrocytes, and hepatocytes (16, 17). The 68 kDa transmembrane type II receptor (IL-1 RII) is expressed on B cells, neutrophils, and bone marrow cells (18). The two IL-1 receptor types show approximately 28% homology in their extracellular domains but differ significantly in that the type II receptor has a cytoplasmic domain of only 29 amino acids (aa), whereas the type I receptor has a 217 aa cytoplasmic domain. IL-1 RII does not appear to signal in response to IL-1 and may function as a decoy receptor that attenuates IL-1 function (19). The IL-1 receptor accessory protein (IL-1 RAcP) associates with IL-1 RI and is required for IL-1 RI signal transduction (20). IL-1ra is a secreted molecule that functions as a competitive inhibitor of IL-1 (21, 22). Soluble forms of both IL-1 RI and IL-1 RII have been detected in human plasma, synovial fluids, and the conditioned media of several human cell lines (23, 24). In addition, IL-1 binding proteins that resemble soluble IL-1 RII are encoded by vaccinia and cowpox viruses (25).

The Quantikine Rat IL-1β Immunoassay is a 4.5 hour solid phase ELISA designed to measure rat IL-1β in cell culture supernates, serum, and plasma. It contains recombinant rat IL-1β and antibodies raised against recombinant rat IL-1β. This immunoassay has been shown to quantitate the recombinant rat IL-1β accurately.

It has been reported that ELISA kits calibrated using mature IL-1β as a standard will detect, but considerably underestimate, the unprocessed IL-1β precursor present in samples (26, 27). While the rat IL-1β precursor has not been tested in this immunoassay kit, it is possible that this kit may also underestimate the rat precursor IL-1β in samples. Nevertheless, in biological samples other than cell lysates, the precursor form of IL-1β (which is not biologically active) is usually not the predominant form of IL-1β. Therefore, results obtained using this kit should provide a useful measure of the levels of active rat IL-1β present in biological fluids.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for rat IL-1β has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any rat IL-1β present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for rat IL-1β 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 rat IL-1β 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.
- 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 #	CATALOG # RLB00	CATALOG # SRLB00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Rat IL-1β Microplate	890539	2 plates	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with polyclonal antibody specific for rat IL-1β.	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.*
Rat IL-1β Standard	890541	3 vials	9 vials	Recombinant rat IL-1β in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconsitution volume.*	Discard within 8 hours of reconstitution. Use a new Standard and Control for each assay.
Rat IL-1β Control	890542	3 vials	9 vials	Recombinant rat IL-1β in a buffered protein base with preservatives; lyophilized. The assayed value of the Control should be within the range specified on the label.	
Rat IL-1β Conjugate	890540	1 vial	3 vials	23 mL/vial of a polyclonal antibody against rat IL-1β conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-21	895215	1 vial	3 vials	12 mL/vial of a buffered protein solution with preservatives.	
Calibrator Diluent RD5Y	895201	2 vials	6 vials	21 mL/vial of a buffered protein solution with preservatives.	
Wash Buffer Concentrate	895003	2 vials	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of a diluted hydrochloric acid solution.	
Plate Sealers	N/A	8 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

RLB00 contains sufficient materials to run ELISAs on two 96 well plates.
SRLB00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PRLB00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

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 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.

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 1000 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 1000 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 may not be suitable for use with this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 3-fold dilution. A suggested 3-fold dilution is 50 µL of sample + 100 µL of Calibrator Diluent RD5Y.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

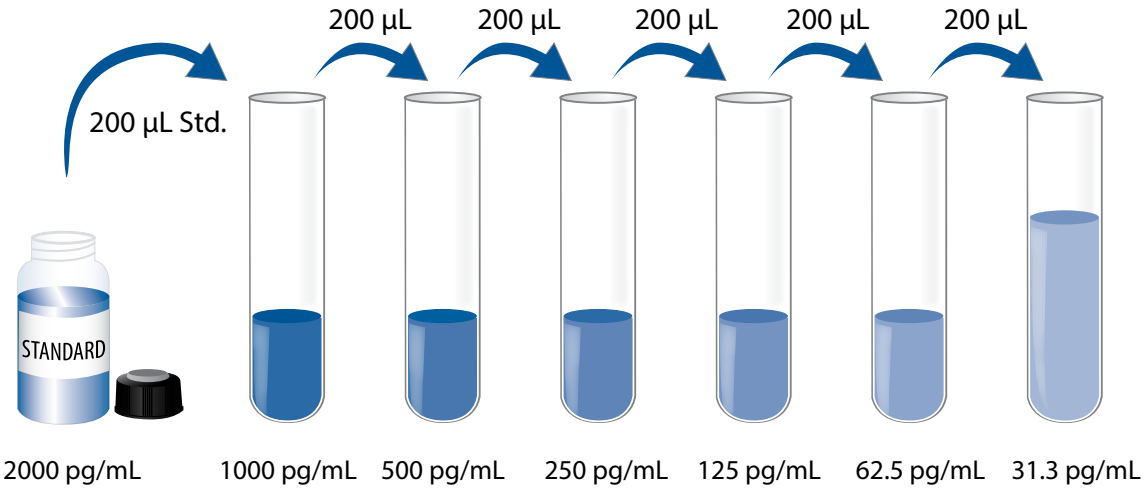
Rat IL-1β Control - Reconstitute the kit Control with 1.0 mL of deionized or distilled water. Assay the Control undiluted.

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 into 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.

Rat IL-1β Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Rat IL-1β Standard with Calibrator Diluent RD5Y. 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 RD5Y 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 Rat IL-1β Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD5Y 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, samples, and control be assayed in duplicate.

1. Prepare all 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 the 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 Rat IL-1β 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

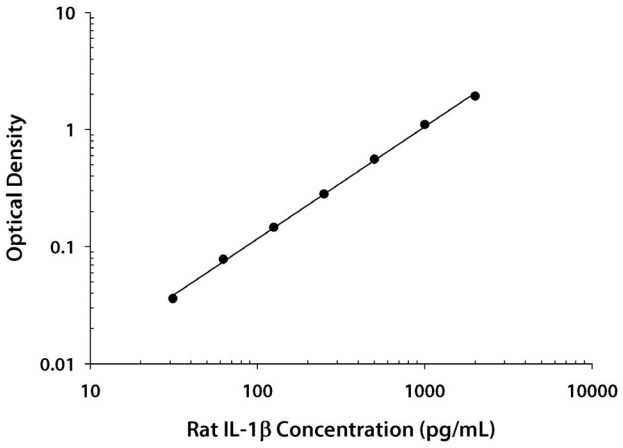
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 rat IL-1β concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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	0.035 0.034	0.034	—
31.3	0.070 0.069	0.070	0.036
62.5	0.111 0.113	0.112	0.078
125	0.177 0.182	0.180	0.146
250	0.322 0.310	0.316	0.282
500	0.592 0.593	0.592	0.558
1000	1.143 1.135	1.139	1.105
2000	1.962 1.960	1.961	1.927

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	83.4	253	610	82.3	249	626
Standard deviation	7.3	9.9	24	4.7	10.0	27
CV (%)	8.8	3.9	3.9	5.7	4.0	4.3

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates (n=5)	100	89-110%
Serum* (n=5)	108	97-120%
EDTA plasma* (n=5)	98	89-111%

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

LINEARITY

To assess the linearity of the assay, samples spiked with various concentrations of rat IL-1β in each matrix were diluted with Calibrator Diluent and then assayed.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=5)
1:2	Average % of Expected	115	107	106
	Range (%)	110-120	104-111	105-106
1:4	Average % of Expected	115	111	105
	Range (%)	112-118	108-116	102-108
1:8	Average % of Expected	110	112	107
	Range (%)	103-113	109-116	102-111
1:16	Average % of Expected	109	106	106
	Range (%)	100-113	90-115	94-113

SENSITIVITY

The minimum detectable dose (MDD) of rat IL-1β 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 a highly purified *E. coli*-expressed recombinant rat IL-1β produced at R&D Systems.

SAMPLE VALUES

Cell Culture Supernates:
Three Wistar rats were given an intraperitoneal injection of 100 µg/kg of LPS. All rats were killed 4 hours after receiving LPS. Lungs and spleens were harvested and broken into individual cells by using a Daunce homogenizer.

Rat lung conditioned media was collected after culturing in 60 mL of RPMI supplemented with 10% fetal bovine serum for 18 hours. The cell culture supernate was assayed for rat IL-1β and measured 1124 pg/mL.

Rat spleen conditioned media was collected after culturing in 60 mL of RPMI supplemented with 10% fetal bovine serum and stimulated with 1 µg/mL of LPS for 18 hours. The cell culture supernate was assayed for rat IL-1β and measured 3752 pg/mL.

Serum - Forty individual rat serum samples were evaluated for detectable levels of rat IL-1β in this assay. Thirty-seven samples read below the lowest standard, 31.3 pg/mL. Three samples read 41, 51, and 123 pg/mL, respectively.

Plasma - Twenty individual rat EDTA plasma samples were evaluated for detectable levels of rat IL-1β in this assay. Nineteen samples read less than the lowest standard, 31.3 pg/mL. One sample read 137 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant rat IL-1β.

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 rat IL-1β control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant rat:
CINC-1
GDNF
IFN-γ
IL-1α
IL-1 RI
IL-1ra
IL-2
IL-4
β-NGF
PDGF-BB
TNF-α

Recombinant mouse:
IL-1α
IL-1ra

Recombinant human:
IL-1 RI
IL-1 RII
IL-1ra

Some cross-reactivity was observed with the following:

Recombinant Factor	Concentration (pg/mL)	Observed Value (pg/mL)	% Cross-reactivity
mouse IL-1β	50,000	871	1.7
human IL-1β	50,000	816	1.6

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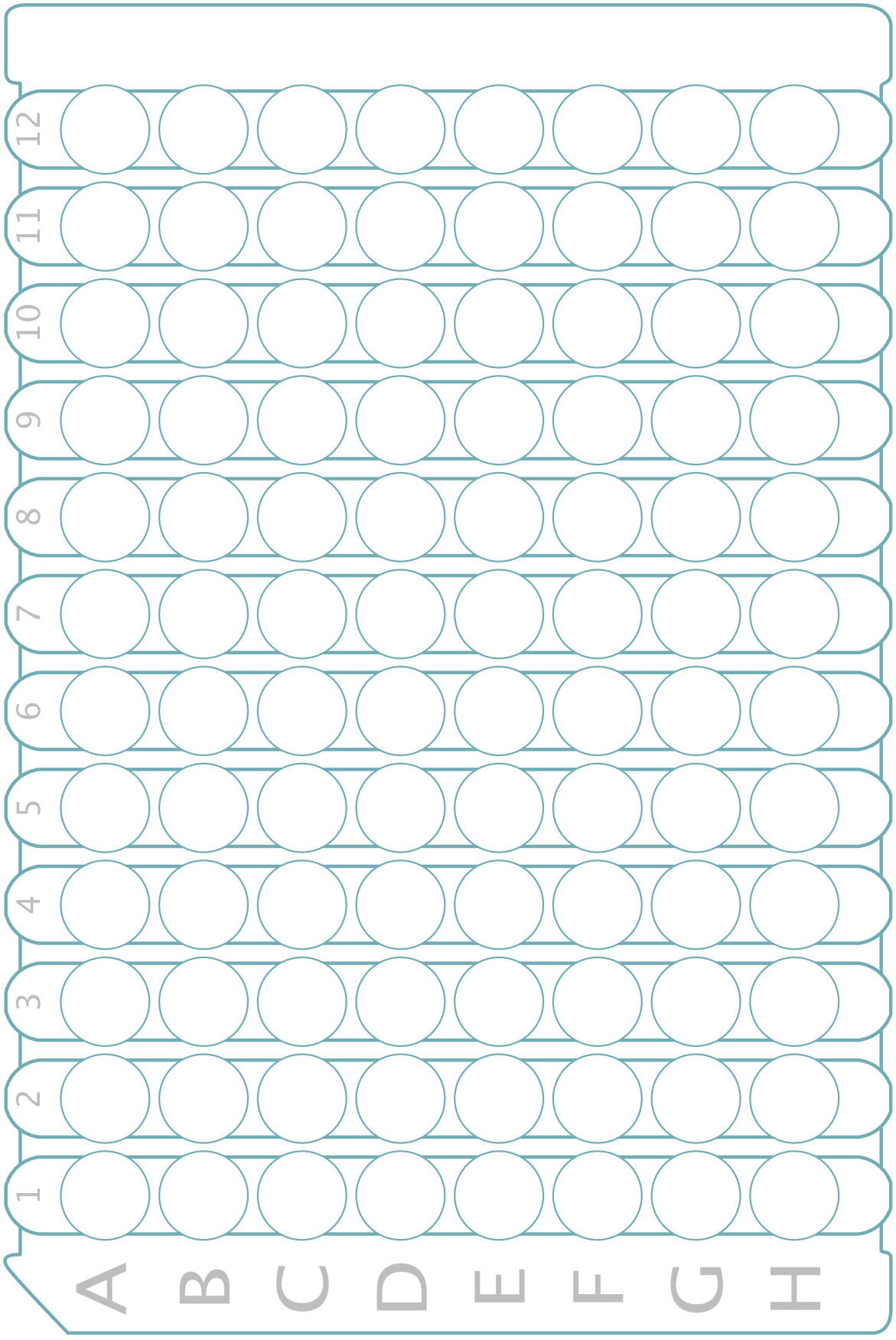
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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