Quantikine® ELISA

Human IL-1 RII Immunoassay

Catalog Number DR1B00

For the quantitative determination of human Interleukin 1 Receptor type II (IL-1 RII) 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

Interleukin 1 (IL-1) is a general name that designates two distinct proteins, IL-1α and IL-1β. Each protein is the product of a separate gene and each protein recognizes the same surface receptors. Although IL-1 production is rapidly induced and generally associated with inflammation, evidence suggests that IL-1 is also temporally upregulated during bone formation and the menstrual cycle, and can be induced in response to nervous system stimulation (1-3).

Both IL-1α and IL-1β are synthesized as 31 kDa precursors that are subsequently cleaved into proteins with molecular weights of approximately 17 kDa (4). IL-1α and IL-1β exert their effects by binding to specific receptors. Two distinct receptors have been identified that bind both forms of IL-1. Common to each is an extracellular immunoglobulin-like (Ig-like) domain that places both IL-1 receptors (IL-1 R) in the type IV cytokine receptor family (5). The first IL-1 receptor (IL-1 RI) is an 80 kDa transmembrane glycoprotein that has been isolated from astrocytes, chondrocytes, endothelium, fibroblasts, keratinocytes, neurons, oocytes, pancreatic β-cells, smooth muscle cells, and T cells (6-12). The second type of IL-1 R (IL-1 RII) is a 60-68 kDa transmembrane glycoprotein which has been found on B cells (7), keratinocytes (8), monocytes (7), neutrophils (13), and T cells (14). The two receptors show approximately 28% homology in their extracellular domains but differ significantly in their cytoplasmic regions where IL-1 RI contains 215 amino acid (aa) residues compared to only 29 for IL-1 RII (15). Depending on species and cell type, the two receptors show differential binding characteristics for IL-1α and IL-1β. In general, IL-1α binds better to the type I receptor, and IL-1β binds better to the type II receptor. Soluble forms of both IL-1 RI and IL-1 RII have been reported in sera (16, 17). The type II soluble receptor is 47 kDa in size and demonstrates high-affinity binding for both IL-1α and IL-1β (18). A 65 kDa glycoprotein has been isolated from murine cells that may form part of an IL-1 R complex as an accessory protein (19). The molecule is 550 aa residues in length, shares 25% aa identity with both IL-1 RI and IL-1 RII, and possesses an Ig-like extracellular domain. This accessory protein forms a complex with IL-1 RI and IL-1 and increases the binding affinity of this receptor for IL-1 (19).

Since IL-1 RII has such a small cytoplasmic domain with no obvious signal transduction capacity, it has been suggested that this receptor acts as a decoy receptor that modulates the activity of IL-1, particularly IL-1β (17, 20). Several observations support this suggestion. Soluble IL-1 RII can bind to the precursor form of IL-1β and inhibit processing to the active form (20). The soluble IL-1 RII shows a greatly decreased affinity for IL-1ra in comparison to the membrane-bound form of the receptor (20) and can act in concert with the IL-1ra to inhibit the activity of IL-1β (21). Thus these two modulators of IL-1 activity do not interfere with each other's actions. Dexamethasone treatment of human PMNs increases the transcription rate of IL-1 RII, prolongs the half-life of the mRNA for IL-1 RII, and augments the expression of the membrane-associated IL-1 RII and the release of the soluble form of IL-1 RII (22). These activities apparently contribute to the immunosuppressive and anti-inflammatory actions of this glucocorticoid. Additionally, the anti-inflammatory cytokine IL-13 also induces the expression and release of IL-1 RII by PMNs (23). These results suggest a role for IL-1 RII, either as a membrane-bound or soluble molecule, in downregulating the overall activity of IL-1 by binding to IL-1 and interfering with its binding to the signal-transducing IL-1 RI.

The Quantikine® Human IL-1 RII Immunoassay is a 3.5 hour solid-phase ELISA designed to measure human IL-1 RII in cell culture supernates, serum, and plasma. It contains *Sf* 21-expressed recombinant human IL-1 RII and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IL-1 RII 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 human IL-1 RII.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-1 RII has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-1 RII present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-1 RII is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-1 RII bound in the initial step. The color development is stopped and the intensity of the color is measured.

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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate 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
Human IL-1 RII Microplate	890426	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-1 RII.	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.*
Human IL-1 RII Conjugate	890427	21 mL of a polyclonal antibody specific for human IL-1 RII conjugated to horseradish peroxidase, with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human IL-1 RII Standard	890428	Recombinant human IL-1 RII in a buffered protein base with preservatives, lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
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	895032	6 mL of 2 N sulfuric 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.
- 100 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.
- Human IL-1 RII Controls (optional; R&D Systems®, Catalog # QC05).

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 and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at approximately 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 , heparin, or citrate as an anticoagulant. Centrifuge for 15 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.

SAMPLE PREPARATION

Serum and plasma samples require at least a 50-fold dilution prior to the assay. A suggested 50-fold dilution is 20 µL of sample + 980 µL of Calibrator Diluent RD5P (1:5).*

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

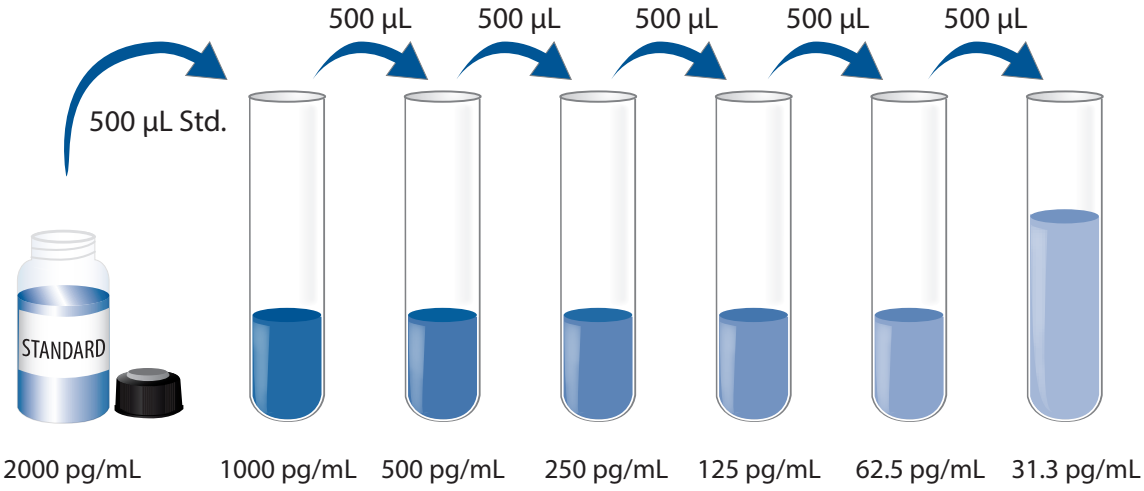
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P Concentrate to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Human IL-1 RII Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-1 RII Standard with Calibrator Diluent RD5P (diluted 1:5). This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of Calibrator Diluent RD5P (diluted 1:5) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human IL-1 RII Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) 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, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards and samples as directed in previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Add 100 µL of Assay Diluent RD1W to each well.
4. Add 100 µL of standard, control, or sample* per well. 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 twice for a total of three 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 by decanting. Invert the plate and blot it against clean paper toweling.
6. Add 200 µL of Human IL-1 RII Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, 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

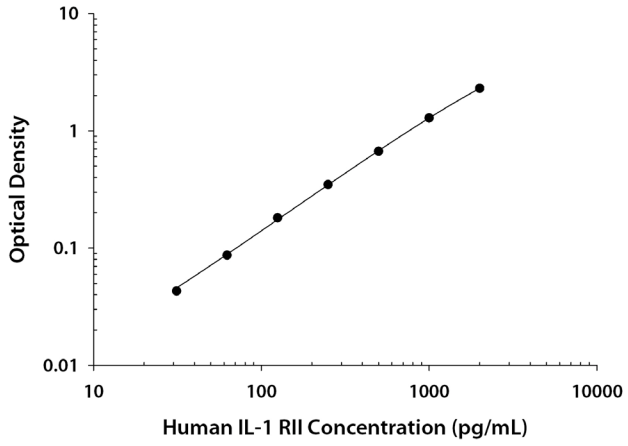
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human IL-1 RII 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 prior to assay, 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	0.020 0.018	0.019	—
31.3	0.061 0.062	0.062	0.043
62.5	0.108 0.105	0.106	0.087
125	0.200 0.200	0.200	0.181
250	0.363 0.374	0.368	0.349
500	0.672 0.703	0.688	0.669
1000	1.332 1.284	1.308	1.289
2000	2.299 2.343	2.321	2.302

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)	72.6	371	721	76.1	371	724
Standard deviation	2.50	7.40	16.9	4.50	17.3	28.5
CV (%)	3.4	2.0	2.3	5.9	4.7	3.9

RECOVERY

The recovery of human IL-1 RII spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	95	86-102%
Serum (n=5)	95	87-106%
EDTA plasma (n=5)	101	95-107%
Heparin plasma (n=5)	100	91-110%
Citrate plasma (n=5)	101	92-109%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human IL-1 RII were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=5)	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)	Citrate plasma (n=5)
1:2	Average % of Expected	102	104	100	100	100
	Range (%)	98-108	102-106	98-103	97-104	96-104
1:4	Average % of Expected	99	106	98	98	100
	Range (%)	93-104	103-109	92-101	94-102	94-105
1:8	Average % of Expected	100	107	98	98	101
	Range (%)	93-106	100-114	93-102	90-104	98-102
1:16	Average % of Expected	95	109	99	100	99
	Range (%)	84-102	92-116	86-104	86-112	82-108

SENSITIVITY

The minimum detectable dose (MDD) of human IL-1 RII is typically less than 10 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 *Sf* 21-expressed recombinant human IL-1 RII produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human IL-1 RII in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=40)	10,787	5828-21,095	2832
EDTA plasma (n=40)	13,354	5468-18,607	3100
Heparin plasma (n=40)	13,715	8406-21,915	3160
Citrate plasma (n=40)	11,300	6015-22,683	3170

Cell Culture Supernates - Human peripheral blood mononuclear cells ($1x10^6$ cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and stimulated with 10 µg/mL PHA. Aliquots of the culture supernates were removed on days 1 and 5 and assayed for levels of human IL-1 RII.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	252	312
Stimulated	168	224

SPECIFICITY

This assay recognizes natural and recombinant human IL-1 RII.

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

Recombinant human:

ANG
AR
CNTF
β-ECGF
EGF
Epo
FGF acidic
FGF basic
FGF-4
FGF-5
FGF-6
FGF-7/KGF
G-CSF
GM-CSF
gp130
GROα
GROβ
GROγ
HB-EGF
HGF
IFN-γ
IGF-I
IGF-II
IL-1α
IL-1β (pro)
IL-1 RI
IL-1ra
IL-2
IL-2 Rα
IL-3
IL-3 Rα
IL-4
IL-4 Rα
IL-5
IL-5 Rα
IL-5 Rβ
IL-6
IL-6 R
IL-7
IL-8
IL-10
IL-11
IL-12
IL-13
KGF
LIF
M-CSF
MCP-1
MIP-1α
MIP-1β
β-NGF
OSM
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
PTN
RANTES
SCF
SLPI
TGF-α
TGF-β1
TGF-β3
LAP (TGF-β1)
TGF-β RII
TNF-α
TNF-β
TNF RI
TNF RII
$VEGF_{165}$

Recombinant mouse:

GM-CSF
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-13
LIF
MIP-1α
MIP-1β
SCF

Recombinant rat:

IL-1ra

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF
human TGF-β1
porcine TGF-β1

Levels of IL-1β up to 7.5 ng/mL (equal to 375 ng/mL in serum samples) do not cause significant interference in this assay.

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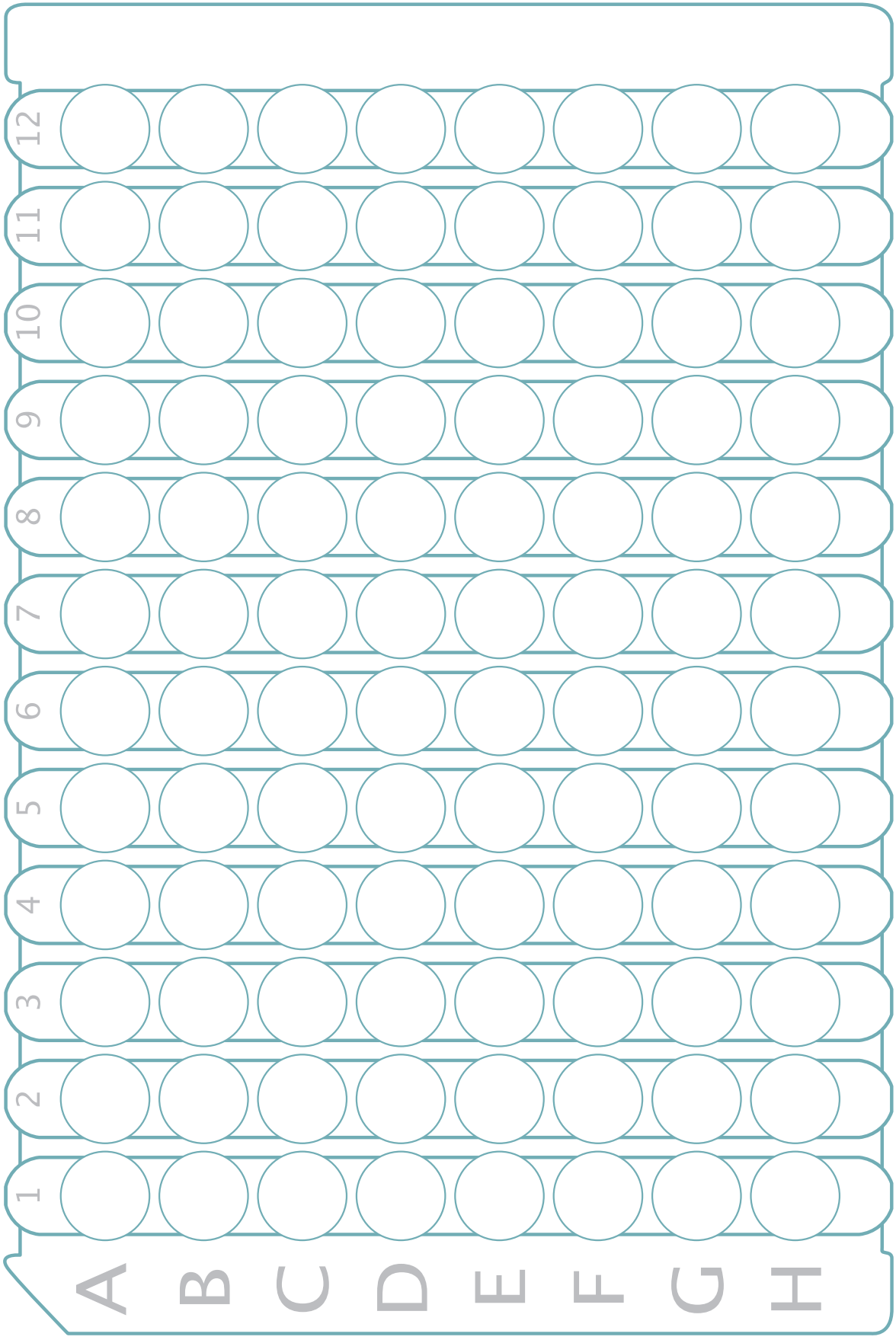
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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