Quantikine™ ELISA

Mouse IL-1α/IL-1F1 Immunoassay

Catalog Number MLA00

For the quantitative determination of mouse Interleukin 1 alpha (IL-1α) 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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Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956
FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

Interleukin 1 alpha (IL-1α) and Interleukin 1 beta (IL-1β) are pleiotropic cytokines that belong to the IL-1 protein family. They bind to the same cell surface receptors and share biological functions. The two proteins have approximately 23% amino acid (aa) sequence homology and both are synthesized as 31 kDa precursors that lack hydrophobic signal peptide sequences. Current evidence suggests that IL-1 proteins may be secreted via non-classical pathways (1-3, 7).

Mouse IL-1α cDNA encodes 270 aa residue pro-IL-1α with a nuclear localization sequence, an N-myristoylation site and three potential N-linked glycosylation sites (1, 2). Pro-IL-1α is primarily localized to the cytosol after synthesis. Some pro-IL-1α is secreted and subsequently cleaved to form the 17 kDa, 154 aa residue mature IL-1α by extracellular proteases such as calpain, a calcium-dependent membrane-associated cysteine protease (4, 5). Pro-IL-1α may be membrane-bound on some cells. Membrane association is mediated either via binding of glycosylated pro-IL-1α to cell-surface lectin, or by interaction of myristoylated pro-IL-1α with plasma membrane phospholipids (2, 6). Unlike pro-IL-1β which is biologically inactive, both pro-IL-1α and mature IL-1α have been shown to bind IL-1 receptor and are biologically active (7, 8). Intracellular pro-IL-1α can also be localized to the nucleus where it plays a role as an intracellular regulator of human endothelial cell proliferation and migration (9, 10). The pro-region of mouse IL-1α is 89% and 76% identical to the rat and human pro-regions, respectively. Within the mature protein, mouse IL-1α shares 79% and 56% aa sequence identity with the rat and human proteins, respectively (11, 12). Cells known to express IL-1α include brown adipocytes (13), keratinocytes (14), monocytes (15), macrophages (16, 17), conjunctival epithelium (17), endothelial cells, and smooth muscle cells (18).

Three type I transmembrane Ig superfamily proteins, IL-1 receptor type I (IL-1 RI), IL-1 RII and IL-1 receptor accessory protein (IL-1 RAcP), are involved in the formation of high affinity cell surface IL-1 receptor complexes (19-21). Both IL-1 RI and IL-1 RII can bind directly with IL-1α/β. IL-1 RAcP does not bind IL-1α/β directly, but interacts with IL-1 RI in the presence of IL-1 to form the high-affinity receptor complex which is required for intracellular signal transduction. IL-1 RAcP also interacts with IL-1 RII to form a non-functional high-affinity receptor complex that does not transduce IL-1 signals. IL-1 RII therefore functions as a decoy receptor that attenuates IL-1α/β functions (22-24). Soluble forms of IL-1 RI, IL-1 RII, and IL-1 RAcP exist (25). Vaccinia and cowpox viruses have also been found to encode IL-1 binding proteins that resemble soluble IL-1 RII (26).

IL-1 possesses a wide variety of biological activities and plays a central role in mediating immune and inflammatory responses. Normal production of IL-1 is critical for hematopoiesis, osteoclast differentiation and initiation of normal host responses to injury and infection (25, 27). Inappropriate production of IL-1 has been implicated in the production of a variety of pathological conditions including sepsis, rheumatoid arthritis, inflammatory bowel disease, acute and chronic myelogenous leukemia, insulin-dependent diabetes mellitus, and atherosclerosis (7, 25).

The Quantikine™ Mouse IL-1α/IL-1F1 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse IL-1α levels in cell culture supernates and serum. It contains *E. coli*-expressed recombinant mouse IL-1α and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse IL-1α accurately. Results obtained using natural mouse IL-1α showed dose response curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse IL-1α.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-1α has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-1α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-1α 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-1α 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.
- It is recommended that 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-1α Microplate	890019	96 well microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-1α.	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-1α Standard	890018	Recombinant mouse IL-1α 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 IL-1α Control	890061	Recombinant mouse IL-1α in a buffered protein base with preservatives; lyophilized. The assayed value of the control should be within the range specified on the label.	
Mouse IL-1α Conjugate	890017	12 mL of a polyclonal antibody specific for mouse IL-1α conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-40	895513	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-16	895302	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
- 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 SDS 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 5-fold dilution prior to assay. A suggested 5-fold dilution is 30 µL of sample + 120 µL of Calibrator Diluent RD5-16.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

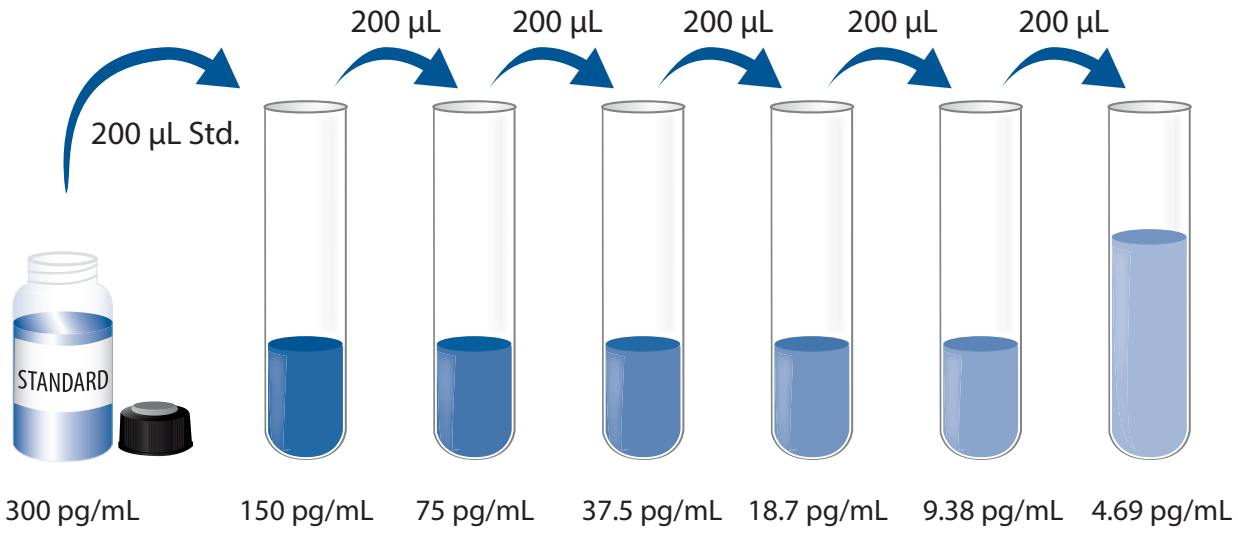
Mouse IL-1α Control - Reconstitute the control with 1 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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 480 mL of 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-1α Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-1α Standard with Calibrator Diluent RD5-16. Do not substitute other diluents. This reconstitution produces a stock solution of 300 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 RD5-16 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse IL-1α Standard (300 pg/mL) serves as the high standard. Calibrator Diluent RD5-16 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, working standards, control, and samples as directed in the previous section.
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 RD1-40 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 IL-1α 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.

*Serum samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

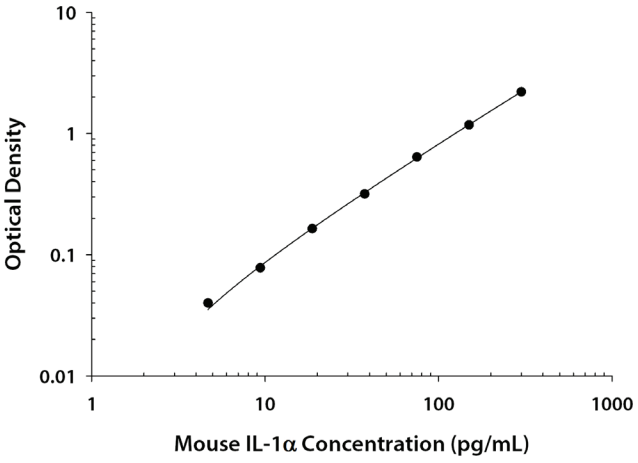
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 IL-1α 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	0.080 0.080	0.080	—
4.69	0.118 0.121	0.120	0.040
9.38	0.157 0.159	0.158	0.078
18.7	0.240 0.247	0.244	0.164
37.5	0.393 0.401	0.397	0.317
75	0.710 0.728	0.719	0.639
150	1.248 1.270	1.259	1.179
300	2.272 2.304	2.288	2.208

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)	17	32	156	18	33	162
Standard deviation	1.5	1.0	3.9	0.9	1.2	7.9
CV (%)	8.8	3.1	2.5	5.0	3.6	4.9

RECOVERY

The recovery of mouse IL-1α spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=5)	102	92-109%
Serum* (n=6)	94	88-103%

*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 mouse IL-1α in each matrix were diluted with calibrator diluent and then 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	Spiked	125	———	———
	1:2	60	62	97%
	1:4	29	31	94%
	1:8	15	16	94%
	1:16	8	8	101%
Serum*	Spiked	144	———	———
	1:2	75	72	104%
	1:4	38	36	106%
	1:8	19	18	106%
	1:16	10	9	111%

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

SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-1α is typically less than 2.5 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 IL-1α produced at R&D Systems®.

The NIBSC/WHO reference preparation 93/672 which was intended as a bioassay standard, was evaluated in this kit. Each ampule contains a nominal 100 ng of recombinant mouse IL-1α and was assigned an arbitrary unitage of 100,000 Units/ampule.

NIBSC/WHO 93/672: 1 Unit of standard = 1 pg of Quantikine™ Mouse IL-1α

SAMPLE VALUES

Serum - Forty samples were evaluated for the presence of IL-1α in this assay.

Number of Samples	Range (pg/mL)
30	< 4.69
9	24-57
1	76

Cell Culture Supernates - Mouse splenocytes (10^6 cells/mL) were cultured for 3 days in RPMI supplemented with 5% fetal bovine serum and stimulated with 5 µg/mL LPS. An aliquot of the cell culture supernate was removed, assayed for mouse IL-1α, and measured 18 pg/mL.

SPECIFICITY

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

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

Recombinant mouse:

C10
Eotaxin
G-CSF
GM-CSF
IFN-γ
IL-1β
IL-1ra
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-12
IL-13
IL-17
IL-18
JE/MCP-1
KC
Leptin
LIF
M-CSF
MIP-1α
MIP-1β
MIP-2
OSM
RANTES
SCF
TNF-α
TNF RI
TNF RII
Tpo
VEGF

Recombinant rat:

IL-1β

Recombinant human:

IL-1α
IL-1β
IL-1 RI
IL-1 RII

Recombinant rat IL-1α cross-reacts approximately 0.78% in this assay.

Recombinant mouse IL-1 RI interferes at concentrations ≥ 5000 pg/mL in this assay.

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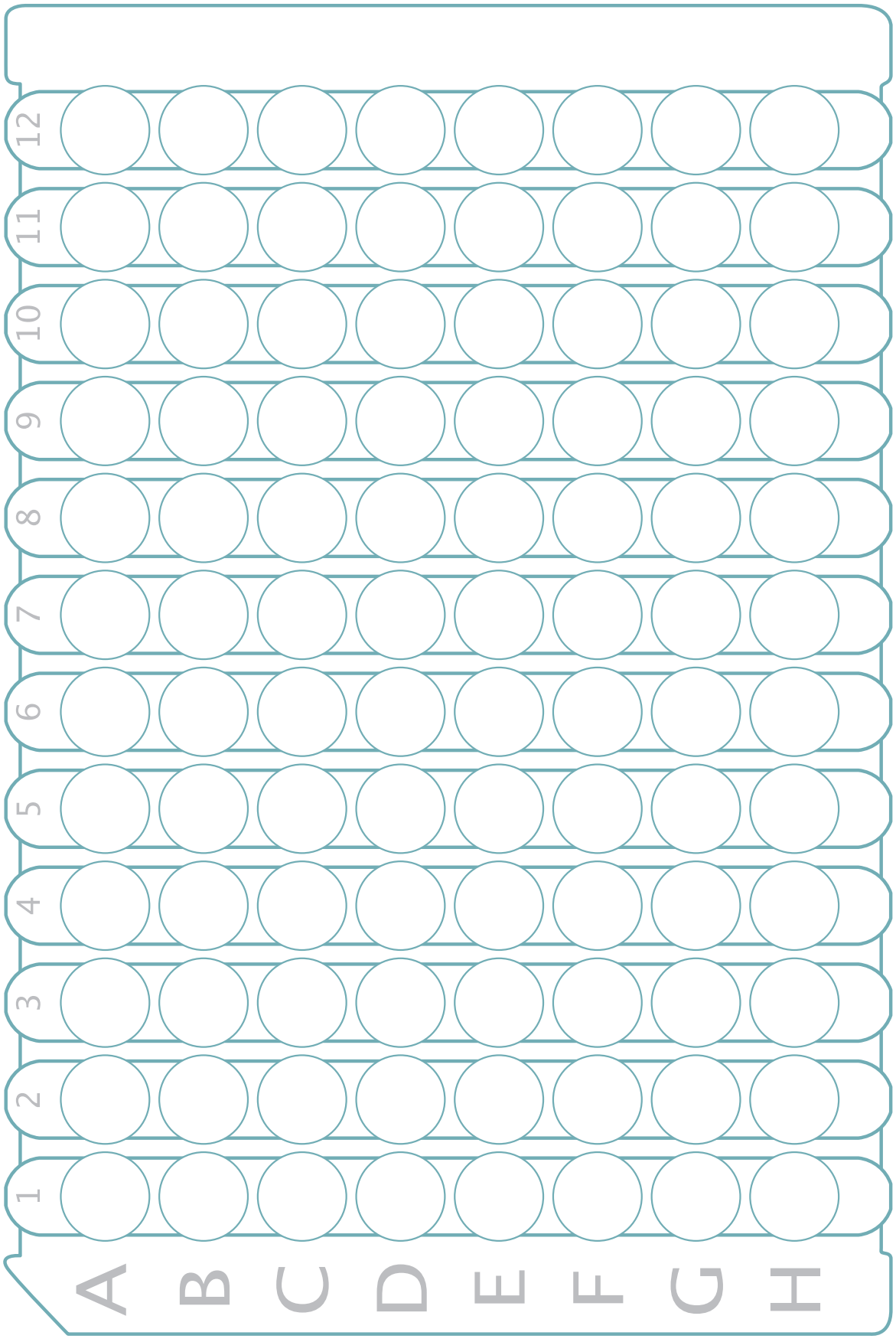
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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