Quantikine® ELISA

Mouse LIF Immunoassay

Catalog Number MLF00

For the quantitative determination of mouse Leukemia Inhibitory Factor (LIF) concentrations in cell culture supernates, serum, and plasma.

Note: 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.

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INTRODUCTION

Mouse Leukemia Inhibitory Factor (LIF) is named for its ability to induce the differentiation of mouse M1 myeloid leukemia cells into macrophages. It is a 40-45 kDa, monomeric glycoprotein involved in a number of reproductive, inflammatory, developmental and regenerative processes (1-4). Three LIF transcripts, LIF-D, LIF-M, and LIF-T, are differentially expressed as a result of alternate first exon and/or promoter usage. LIF-D is synthesized as a 203 amino acid (aa) precursor that contains a 23 aa signal sequence and an α-helical 180 aa mature segment (5, 6). This molecule is highly glycosylated, and depending on the site(s) used for carbohydrate attachment, the presence of sugar may impact LIF's bioactivity (7). Notably, mannose-6-P, one of the potential glycosylating sugars, will likely regulate the bioavailability of LIF via its binding to IGF-II receptors on target cells (8). LIF-T is a 17 kDa, 158 aa intracellular molecule that shows truncation of the first 22 aa of the mature LIF-D protein. Within helix B of LIF-T lies a stretch of 35 aa that is leucine-rich and closely resembles leucine-zipper transcription factors (9-11). It is assumed that this molecule operates within the nucleus of the cell. LIF-M is reported to occur extracellularly and to bind to extracellular matrix (10, 12, 13). The function(s) of LIF-M and -T are poorly understood. Mature mouse LIF-D shares 79%, 78%, 74%, and 92% aa identity to human, porcine, sheep, and rat LIF-D, respectively (14-17). Mammalian cells known to express LIF include macrophages and olfactory neurons (18), parasympathetic neurons (19), eosinophils (20), colonic epithelium (19, 21), $CD4^+$ T cells (22), Schwann cells (23), adrenal cortex parenchymal cells (24), fibroblasts (25, 26), vascular and visceral smooth muscle (25), keratinocytes (27), astrocytes (28), mast cells (29), endothelial cells (30), skeletal muscle (31), plus pituitary corticotrophs and somatotrophs (32).

The functional receptor for LIF is composed of a 190 kDa ligand-binding subunit termed LIF R, and a 130 kDa signal transducing subunit termed gp130 (33-36). LIF R is a 1050 aa type 1 transmembrane glycoprotein that is a member of the hematopoietic receptor superfamily. It contains a 785 aa extracellular region, a 25 aa transmembrane segment, and a 240 aa cytoplasmic domain. The extracellular region is characterized by the presence of two cytokine-binding domains (CBD) that are separated by an Ig-like domain. The N-terminal CBD (D1) is involved with LIF binding. The Ig-module contributes to LIF binding and species specificity. The transmembrane-proximal CBD is involved in the maintenance of proper D1-Ig 3D configuration and gp130 interaction (37-40). Following LIF binding to LIF R, gp130 is recruited to the complex forming a signaling receptor. Although the functional receptor for LIF is considered heterotrimeric, there is evidence to suggest that it is actually heterotetrameric (40). In addition to membrane-bound LIF R, soluble LIF R also occurs constitutively as a liver-produced protein (41).

LIF is pleiotropic in its effects. In reproduction, LIF is suggested to play a role in the initiation and maintenance of early pregnancy. In the embryo, LIF maintains the inner cell mass as an undifferentiated collection of cells. This guarantees a critical mass of embryonic stem cells, for subsequent embryonic differentiation (2). It also likely plays a role in endometrial remodeling and influences the activities of uterine immune cells (1, 4). In the pituitary, LIF induces POMC expression and ACTH secretion and promotes fetal corticotroph development (1). During inflammation, LIF has been suggested to be both proinflammatory and anti-inflammatory and appears to play a key role in neural injury and regeneration. In particular, LIF promotes wound resolution via white cells and may stimulate neuronal regrowth in certain situations (3).

The Quantikine Mouse LIF Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse LIF levels in cell culture supernates, serum, and EDTA plasma. It contains *E. coli*-expressed recombinant mouse LIF and antibodies raised against the recombinant factor. Results obtained for naturally occurring mouse LIF 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 of natural mouse LIF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse LIF has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse LIF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse LIF 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 mouse LIF 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.
- 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 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.

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 LIF Microplate	892298	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse LIF.	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 LIF Conjugate	892299	12 mL of a polyclonal antibody specific for mouse LIF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse LIF Standard	892304	Recombinant mouse LIF in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse LIF Control	892305	Recombinant mouse LIF in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Assay Diluent RD1W	895038	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.

For Sample pre-treatment:

- Glacial acetic acid.
- HEPES, free acid.
- Sodium hydroxide.
- 250 mL graduated cylinder.

PRECAUTIONS

Some components of this kit contain sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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. 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 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 as an anticoagulant. Centrifuge 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: *Heparin and citrate plasma have not been validated for use in this assay.*

ACID PRE-TREATMENT REAGENT PREPARATION

Caution: *Wear protective clothing and safety glasses during preparation or use of these reagents. Refer to the appropriate MSDS before use.*

2.5 N Acetic Acid (250 mL) - To 100 mL of deionized or distilled water, slowly add 35.9 mL of Glacial Acetic Acid. Mix well. Bring final volume to 250 mL with deionized or distilled water.

2.7 N NaOH/1 M HEPES (250 mL) - To 140 mL of deionized water, add 67.5 mL of 10 N NaOH. Mix well. Add 59.5 g HEPES. Mix well. Bring final volume to 250 mL with deionized or distilled water.

SAMPLE PREPARATION

To maximize recovery, serum and plasma samples require pre-treatment with 2.5 N acetic acid and then neutralization with 2.7 N NaOH/1M HEPES prior to being assayed. Add 50 µL of sample + 50 µL of 2.5 N acetic acid. Mix well and incubate for 10 minutes at room temperature. Neutralize with 30 µL of 2.7 N NaOH/1M HEPES. Mix well and incubate for at least 5 minutes at room temperature. This process results in a 2.6-fold dilution of the original factor.

After each new lot of acidification and neutralization reagents is prepared, ensure that the pH of several samples is between 7.2-7.6. Adjust the volume and corresponding dilution factor of the neutralization reagent as needed.

Note: *Cell culture supernate samples do not require pre-treatment.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

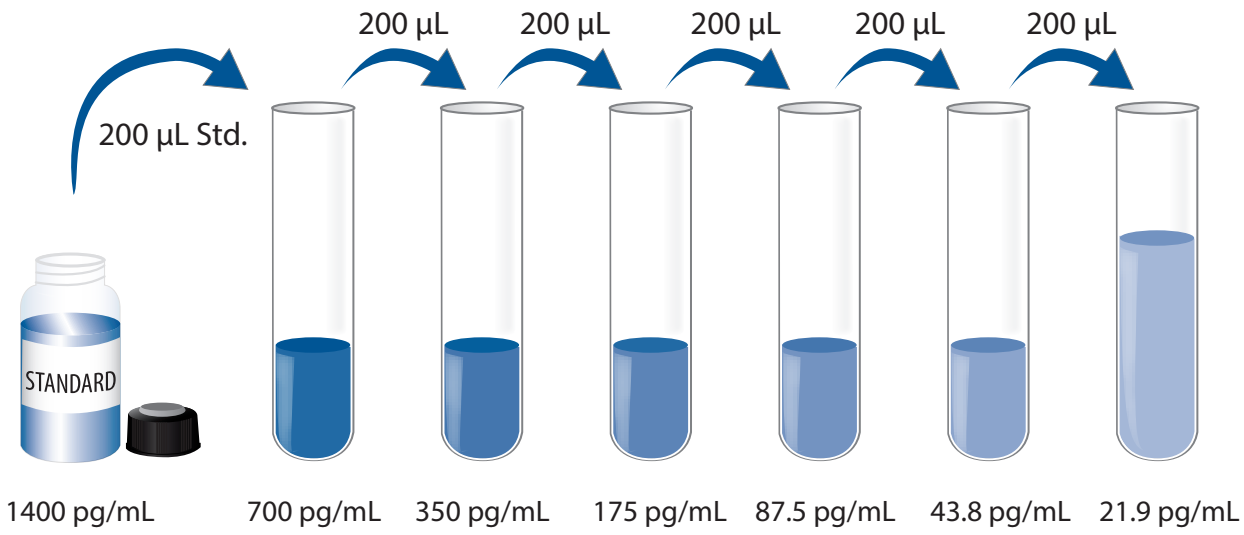
Mouse LIF Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

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 LIF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse LIF Standard with Calibrator Diluent RD5-16. Do not substitute other diluents. This reconstitution produces a stock solution of 1400 pg/mL. Mix the standard to ensure complete reconstitution and 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 standard stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse LIF Standard (1400 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, 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 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 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 Mouse LIF 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 and plasma samples require acid pre-treatment. See Sample Preparation section.

CALCULATION OF RESULTS

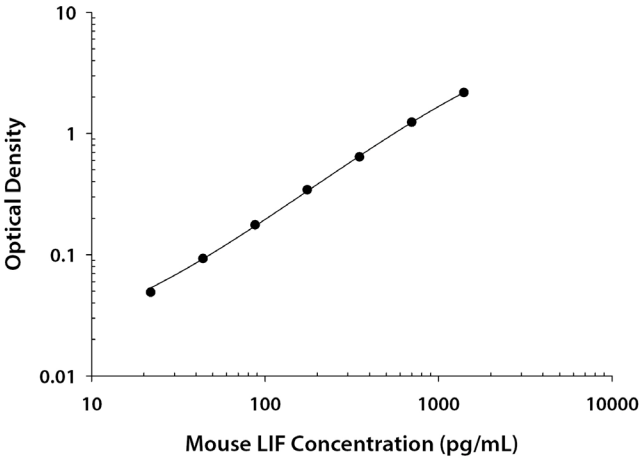
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

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 LIF 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.

Because serum and plasma samples have been pretreated and 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	0.040 0.043	0.042	—
21.9	0.091 0.091	0.091	0.049
43.8	0.134 0.136	0.135	0.093
87.5	0.217 0.218	0.218	0.176
175	0.380 0.390	0.385	0.343
350	0.681 0.682	0.682	0.640
700	1.269 1.295	1.282	1.240
1400	2.220 2.222	2.221	2.179

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)	80	278	651	87	278	656
Standard deviation	4.1	24	50	5.0	12	35
CV (%)	5.1	8.6	7.7	5.7	4.3	5.3

RECOVERY

The recovery of mouse LIF spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=6)	98	81-108%
Serum* (n=5)	96	90-108%
EDTA plasma* (n=6)	97	87-110%

*Samples were prepared according to the Acid Pre-Treatment Sample Preparation section prior to assay.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse LIF were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=6)	Serum* (n=6)	EDTA plasma* (n=6)
1:2	Average % of Expected	93	102	101
	Range (%)	90-97	100-106	97-106
1:4	Average % of Expected	90	106	103
	Range (%)	88-93	102-109	98-107
1:8	Average % of Expected	94	111	111
	Range (%)	90-98	105-118	103-114
1:16	Average % of Expected	94	97	103
	Range (%)	85-98	90-103	90-109

*Samples were prepared according to the Acid Pre-Treatment Sample Preparation section prior to assay.

SENSITIVITY

Twelve assays were evaluated and the minimum detectable dose (MDD) of mouse LIF ranged from 1.34-3.13 pg/mL. The mean MDD was 1.94 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 mouse LIF.

SAMPLE VALUES

Serum/Plasma - Twenty samples were evaluated for detectable levels of mouse LIF in this assay. All samples measured less than the lowest standard, 21.9 pg/mL.

Cell Culture Supernates:
Lungs from one mouse were cultured for 7 days in 50 mL RPMI supplemented with 10% fetal calf serum. An aliquot of the cell culture supernate was removed, assayed for mouse LIF, and measured 471 pg/mL.

EL4.IL-2 mouse lymphoblast cells (1 x 10^6 cells/mL) were cultured for 3 days in 100 mL DMEM supplemented with 10% fetal calf serum and stimulated with 10 µg/mL PHA and 10 ng/mL PMA. An aliquot of the cell culture supernate was removed, assayed for mouse LIF, and measured 125 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse LIF.

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

Recombinant mouse:

C10
Eotaxin
E-Selectin
Fas Ligand
Follistatin
G-CSF
GM-CSF
gp130
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 p70
IL-13
IL-17
IL-18
JE/MCP-1
L-Selectin
M-CSF
MIP-1α
MIP-1β
MIP-1γ
MIP-2
OPG
OSM
OSM Rβ
PlGF-2
RANK
RANK Ligand
RANTES
SCF
TARC
TGF-β RII
TIMP-1
TNF-α
TNF RI
TNF RII
Tpo
VCAM-1
VEGF
VEGF R1
VEGF R2
VEGF R3

Recombinant human:

LIF

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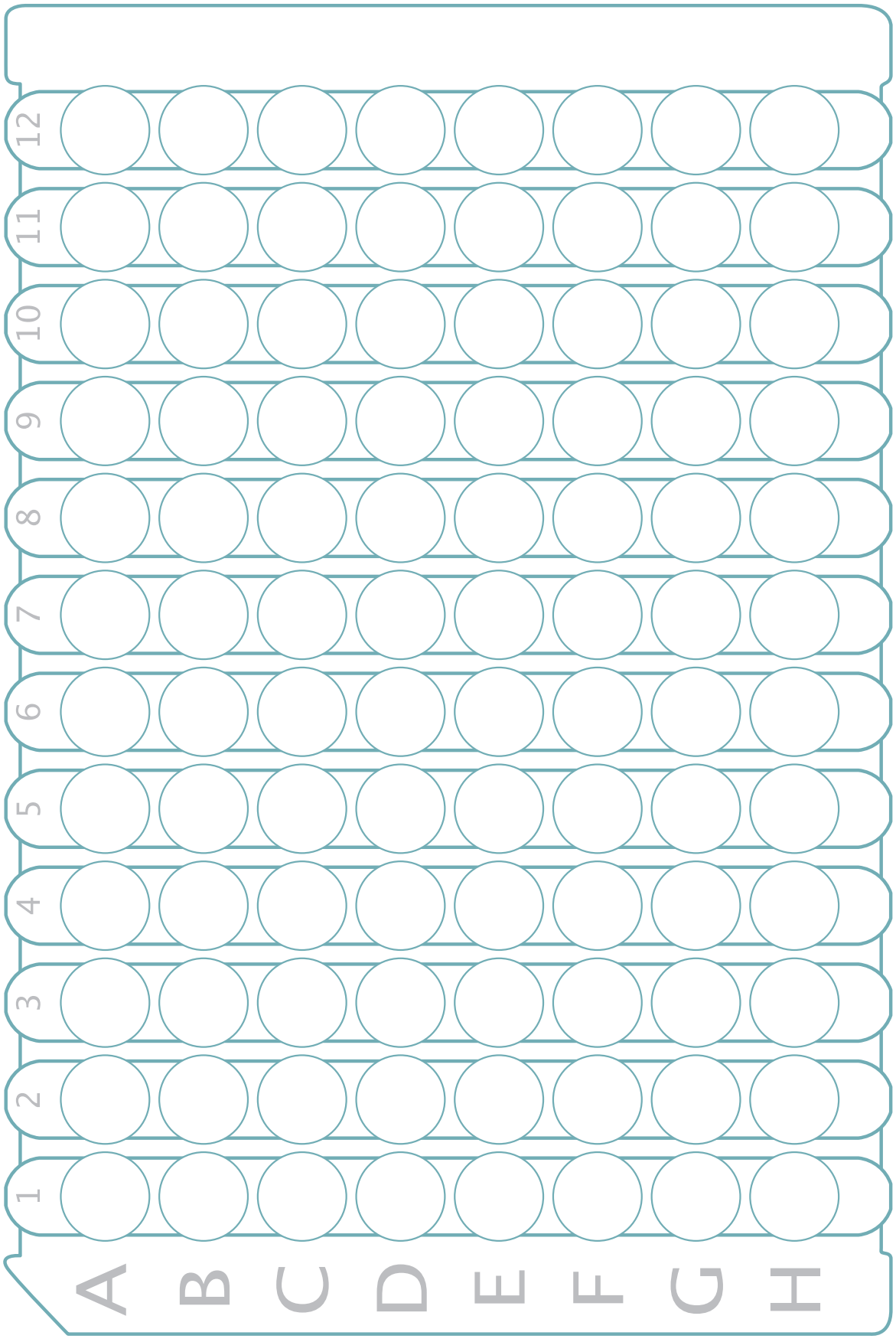
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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