Quantikine® ELISA

Mouse CXCL5/LIX Immunoassay

Catalog Number MX000

For the quantitative determination of mouse LPS-induced CXC chemokine (LIX) concentrations in cell culture supernates, tissue lysates, serum, platelet-poor plasma, and urine.

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 CXCL5/LIX [lipopolysaccharide (LPS)-induced CXC chemokine], also called CXCL5/6 and previously GCP-2, is a member of the ELR+ group of 7-9 kDa neutrophil and monocyte chemotactic proteins in the CXCL cytokine family (1). This group contains the amino acid (aa) motif ELR immediately N-terminal to the CXC motif. ELR+ chemokines also include mouse CXCL1-3 and 15 (KC, MIP-2, DCIP-1, and lungkine, respectively) and human CXCL1-3 and 5-8 (GROα, GROβ, GROγ, ENA-78, GCP-2, NAP-2, and IL-8, respectively) (1-4). Mouse LIX cDNA encodes 132 aa including a 40 aa signal peptide and a 92 aa mature protein that has a longer C-terminus than other ELR+ cytokines (5). The first 78 aa of mature LIX shares 61% and 55% aa identity with human CXCL6/GCP-2 and CXCL5/ENA-78, respectively. Its activity is similar, but not identical, to these human chemokines (1, 5). In the mouse, CXCL1/KC and CXCL2/MIP-2 expression and function may overlap with that of LIX (1, 6-10).

LIX can be produced by a variety of cells including fibroblasts, epithelial cells (including alveolar type II epithelia), endothelial cells, platelets, cardiac myocytes, osteoblasts, oligodendrocytes, and adipose-resident macrophages (1, 4-8, 11-16). It is mainly produced when induced by LPS, IL-17, and/or TNF-α (1, 5-7, 11, 15). LIX can also be stored within specialized granules, such as platelet α-granules and endothelial cytoplasmic granules, but not Weibel-Palade bodies (6, 13). Endotoxemia increases LIX expression, especially in the heart, but also in the lung, spleen, and liver (8). LIX is downregulated by glucocorticoids and is considered a glucocorticoid-attenuated response gene or GARG (5, 8). It can also be downregulated by IL-10 and viral proteins (12, 17). Natural mouse LIX includes short forms that may be N-terminally cleaved by MMP-1, -2, -8, -9, -12, or -13, and/or C-terminally cleaved by MMP-1, -8, -9, or -12 (1-3, 18, 19). Unlike other ELR$^+$ chemokines, it is not cleaved within the ELR motif, and short forms show enhanced activity as compared to the full-length form (1-3, 18, 19).

LIX activities are mainly mediated by its receptor CXCR2, which is expressed on neutrophils, mast cells and macrophages (9, 16, 17). Unlike other ELR$^+$ chemokines, mouse LIX and human IL-8 can also signal through CXCR1 (2, 17). LIX is the most potent mouse neutrophil chemoattractant and activator (1-4, 7-9, 11, 13, 14). CXCR2-expressing cells treated with LIX show activation of NFκB signaling pathways, resulting in increased production of inflammatory cytokines such as IL-1β and TNF-α (9, 17). TNF-α produced as a result of endotoxemia or ischemia-reperfusion can, in turn, induce cardiomyocyte production of LIX, influx of neutrophils, and impedance of cardiac contractile activity (7, 14). LIX participates in the induction of LPS-induced acute lung injury and in lung ischemia-induced angiogenesis (10, 11). LIX protects neurons from apoptosis, and its downregulation by viral proteins is thought to allow neuronal apoptotic death (12). It is thought to play a protective role in inflammatory bone loss (15). High expression of LIX in obese white adipose tissue is thought to contribute to insulin resistance (16). LIX also binds the erythrocyte receptor, DARC (Duffy Antigen Receptor for Chemokines) and is reported to impair chemokine scavenging by DARC (13).

The Quantikine Mouse CXCL5/LIX Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse LIX levels in cell culture supernates, tissue lysates, serum, platelet-poor plasma, and urine. It contains *E. coli*-expressed recombinant mouse LIX and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse LIX accurately. Results obtained using natural mouse LIX showed dose-response curves that were parallel to the standard curves obtained using the recombinant kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse LIX.

PRINCIPLE OF THE ASSAY

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

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.

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 LIX Microplate	894295	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse LIX.	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 LIX Standard	894297	2 vials (2 ng/vial) of recombinant mouse LIX in a buffered protein base with preservatives; lyophilized.	Discard after use. Prepare fresh for each use.
Mouse LIX Control	894298	2 vials of recombinant mouse LIX in a buffered protein base with preservatives; lyophilized. The concentration range of mouse LIX after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.	
Mouse LIX Conjugate	894296	12 mL of a polyclonal antibody specific for mouse LIX 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 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.
- **Polypropylene** test tubes for dilution of standards and samples.

If using tissue lysate samples, the following are also required:

- Cell Lysis Buffer 2 (R&D Systems, Catalog # 895347).
- PBS

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.

Tissue Lysates - Prior to assay, tissues must be lysed according to the directions in the Sample Values section.

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.

Platelet-poor Plasma - Collect plasma on ice using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. An additional centrifugation step of the plasma at 10,000 x g for 10 minutes at 2-8 °C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

LIX is present in neutrophil granules and is released upon neutrophil exposure to activated platelets. Therefore, to measure circulating levels of LIX, platelet-free plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical Laboratory and Standards Institute (CLSI), result in incomplete removal of platelets from blood. This will cause variable and irreproducible results for assays of factors released by platelet activation.

Urine - Collect urine using a metabolic cage. Remove any particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional precipitates that may appear after storage.

SAMPLE PREPARATION

Serum samples require a 20-fold dilution. A suggested 20-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent RD5-16.

Platelet-poor plasma and urine samples require a 4-fold dilution. A suggested 4-fold dilution is 40 µL of sample + 120 µL of Calibrator Diluent RD5-16.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

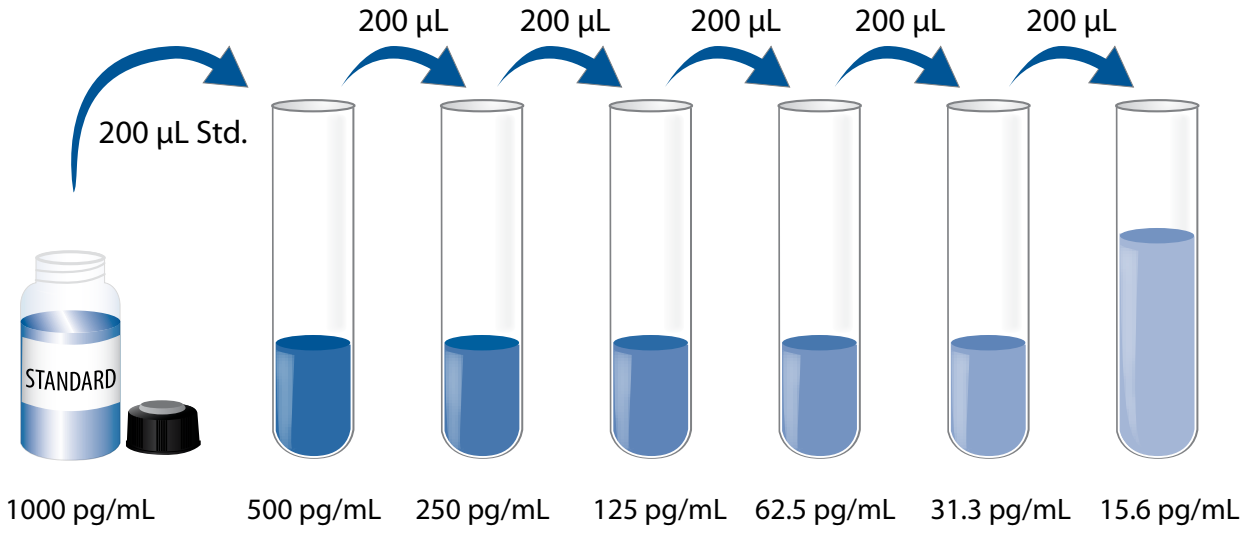
Mouse LIX Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

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 LIX Standard - Reconstitute the Mouse LIX Standard with 2.0 mL of Calibrator Diluent RD5-16. This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-16 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 Mouse LIX Standard (1000 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 all reagents, working standards, 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. 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. **Allow the plate to soak for 30 seconds per wash.** 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 LIX 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 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

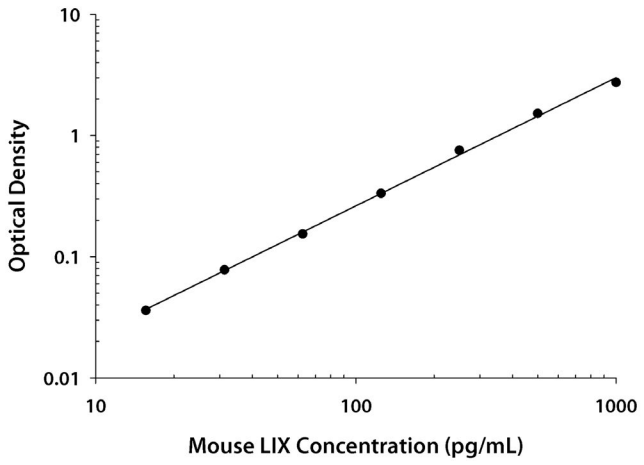
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse LIX 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	0.006 0.006	0.006	—
15.6	0.041 0.043	0.042	0.036
31.3	0.083 0.084	0.084	0.078
62.5	0.155 0.164	0.160	0.154
125	0.334 0.343	0.339	0.333
250	0.728 0.791	0.760	0.754
500	1.495 1.558	1.527	1.521
1000	2.729 2.772	2.751	2.745

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)	42.7	133	407	41.8	120	393
Standard deviation	1.72	5.93	10.9	2.81	4.64	20.3
CV (%)	4.0	4.5	2.7	6.7	3.9	5.2

RECOVERY

The recovery of mouse LIX spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture samples (n=4)	114	105-120%
Tissue lysates (n=4)	100	87-113%
Serum* (n=4)	87	82-94%
Platelet-poor EDTA plasma* (n=4)	100	92-109%
Platelet-poor heparin plasma* (n=4)	91	85-99%
Urine (n=4)	91	84-96%

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

SENSITIVITY

Fifty assays were evaluated and the minimum detectable dose (MDD) of mouse LIX ranged from 0.347-1.74 pg/mL. The mean MDD was 0.817 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*-derived recombinant mouse LIX produced at R&D Systems.

LINEARITY

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

		Cell culture samples (n=4)	Tissue lysates (n=4)	Serum* (n=4)	Platelet-poor		Urine* (n=4)
					EDTA plasma* (n=4)	Heparin plasma* (n=4)	
1:2	Average % of Expected	108	111	99	98	100	99
	Range (%)	100-116	103-114	91-106	94-104	95-108	96-103
1:4	Average % of Expected	108	103	97	91	98	93
	Range (%)	101-117	85-114	88-108	85-93	86-116	84-104
1:8	Average % of Expected	111	95	93	87	96	91
	Range (%)	101-120	80-104	85-105	81-99	85-104	83-100
1:16	Average % of Expected	102	102	93	88	99	91
	Range (%)	88-116	93-111	88-106	88-89	99-100	81-98

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

SAMPLE VALUES

Serum/Plasma/Urine - Samples were evaluated for the presence of mouse LIX in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	11,307	6525-15,418	2868
Platelet-poor EDTA plasma (n=10)	2968	342-6709	1978
Platelet-poor heparin plasma (n=10)	3047	1092-8781	2250

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Urine (n=16)	318	38	ND-1056

ND=Non-detectable

Cell Culture Supernates - Organs from mice were removed, rinsed in PBS, and kept on ice. The organs were then homogenized using a tissue homogenizer and cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 1 µg/mL LPS for 3 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse LIX.

Tissue Type	Unstimulated (pg/mL)	Stimulated (pg/mL)
Lung	266	426
Spleen	341	626

Tissue Lysates - Organs from mice were rinsed with PBS and homogenized with a tissue homogenizer in PBS. An equal volume of Cell Lysis Buffer 2 was added and tissues were lysed at room temperature for 30 minutes with gentle agitation. Debris was then removed by centrifugation. Aliquots of the lysates were removed and assayed for levels of mouse LIX.

Tissue Type	(pg/mL)
Lung	1125
Spleen	1862

SPECIFICITY

This assay recognizes natural and recombinant mouse LIX.

The factors listed below were prepared at 10 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 10 ng/mL in a mid-range recombinant mouse LIX control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:
CXCL1/KC
CXCL2/MIP-2

Recombinant rat:
CXCL5/LIX

Recombinant human:
CXCL5/ENA-78
CXCL6/GCP-2

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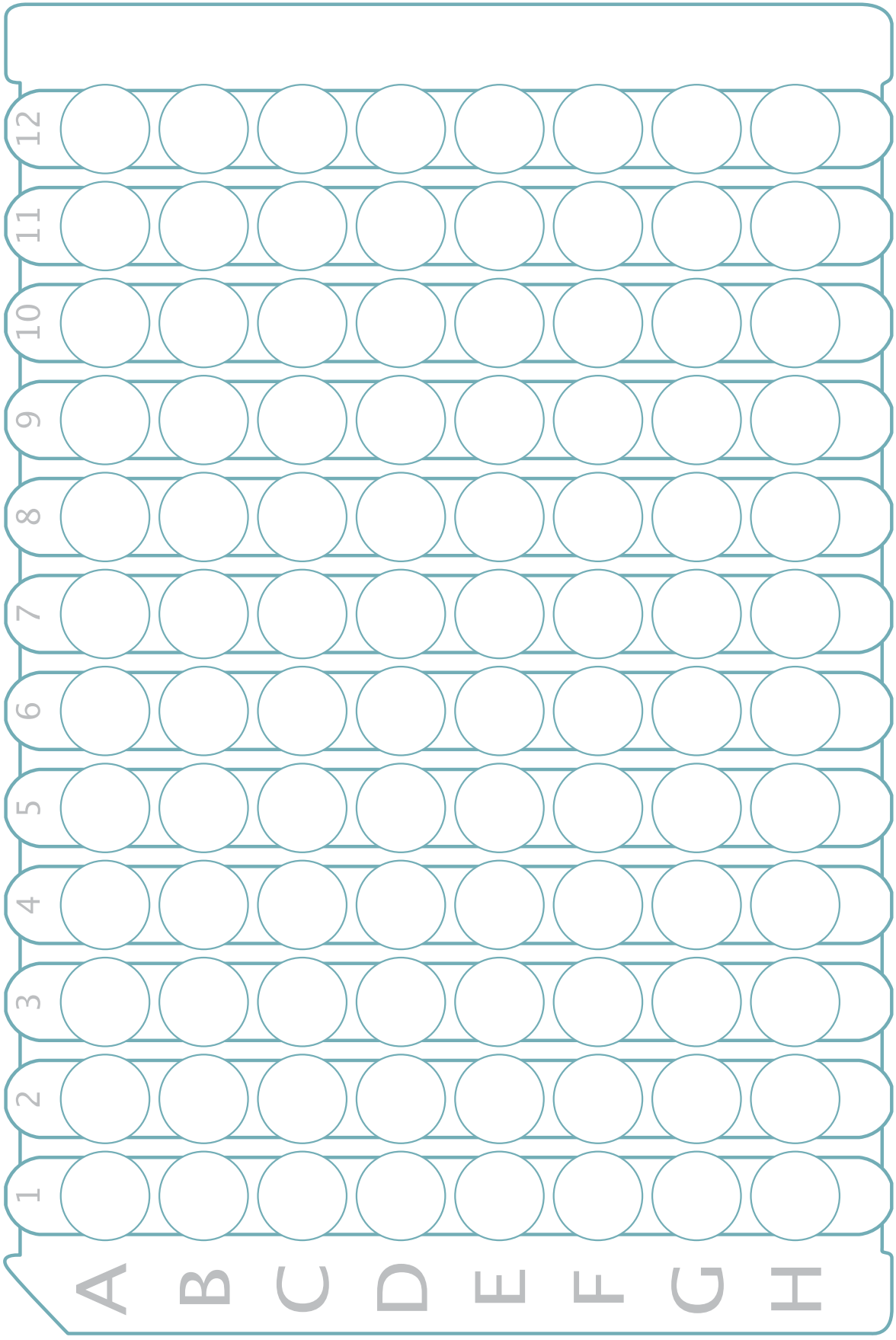
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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