Quantikine® ELISA

Mouse Chitinase 3-like 3/ECF-L Immunoassay

Catalog Number MC3L30

For the quantitative determination of Chitinase 3-like 3 (CHI3L3) concentrations in cell culture supernates, cell lysates, 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.

TABLE OF CONTENTS

SECTION	PAGE
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	2
TECHNICAL HINTS	2
MATERIALS PROVIDED & STORAGE CONDITIONS	3
OTHER SUPPLIES REQUIRED	4
PRECAUTIONS	4
SAMPLE COLLECTION & STORAGE	4
SAMPLE PREPARATION	4
REAGENT PREPARATION	5
ASSAY PROCEDURE	6
CALCULATION OF RESULTS	7
TYPICAL DATA	7
PRECISION	8
RECOVERY	8
LINEARITY	8
SENSITIVITY	9
CALIBRATION	9
SAMPLE VALUES	9
SPECIFICITY	10
REFERENCES	11
PLATE LAYOUT	12

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INTRODUCTION

Mouse Chitinase 3-like 3 (CHI3L3), also known as ECF-L (eosinophil chemotactic factor-lymphocyte) or Ym1, is a secreted ~45 kDa glycoprotein that is a member of the glycoside hydrolase family 18 (chitinase-like) proteins (1-3). Mouse and rat CHI3L3 share 80% amino acid (aa) sequence identity. There is no ortholog in humans; the most related human protein is AMCase, which shares 56% aa sequence identity (3). Mice also express CHI3L4 (also known as Ym2), which shares 90% aa sequence identity with CHI3L3 (2, 4, 5). Analysis of RNA expression patterns of the two mouse proteins indicates partial, but not complete, overlap in cell types and tissues (2, 5). CHI3L3 is primarily secreted by alveolar and peritoneal macrophages during inflammation, such as that following nematode infection and airway hyper-responsiveness (5-11). It is considered a marker for alternatively activated macrophages (5-11). These macrophages are activated by Th2 cytokines, especially IL-4 and sometimes IL-13, and are anti-inflammatory and promote wound healing (5-13). CHI3L3 has also been reported to be expressed in bone marrow myeloid precursors (5), activated microglia (5), testicular macrophages (13), bone marrow-derived immature and connective tissue-type mast cells (12), neutrophil granules in bone marrow, peritoneum and spleen red pulp (2, 14), and other antigen presenting cells such as B cells and dendritic cells (DC) (4, 9, 15). Statins (cholesterol-lowering drugs) are reported to upregulate CHI3L3 expression in DC and promote Th2 responses (15).

In bone marrow erythroblastic islands, or in mice with abnormalities such as SHP-1 deficiency (motheaten), heparinase deficiency, or p47phox (NADPH oxidase) deficiency, CHI3L3 can form crystals in the macrophage cytoplasm (2, 14, 16, 17). This property has been used to aid purification and structural analysis of the protein. Reports differ as to its enzymatic and binding properties, but later reports indicate that chitin binding or degradation is weak or absent (5, 6, 8, 14, 16). Although originally identified as an eosinophil chemotactic protein, significant chemotactic activity is unlikely (1, 5, 10). Binding of GlcN polymers and heparin/heparan sulfates has been identified by some, but other testing and structural determinations indicate that these interactions may also be weak (3, 8, 14, 18). One group has identified an activity as a cofactor with RANKL or vitamin D in stimulating osteoclast differentiation (19). CHI3L3 is proposed to act via promoting cell-cell interaction due to upregulation of the adhesion molecules LFA-1 (integrin $\alpha_L\beta_2$) and ICAM-1 (20).

The Quantikine Mouse Chitinase 3-like 3/ECF-L immunoassay is a 4.5 hour solid phase ELISA designed to measure CHI3L3 in cell culture supernates, cell lysates, serum, and plasma. It contains NS0-expressed recombinant mouse CHI3L3 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural CHI3L3 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 naturally occurring mouse CHI3L3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse CHI3L3 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any CHI3L3 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse CHI3L3 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 CHI3L3 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.
- 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.
- 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 CHI3L3 Microplate	893839	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse CHI3L3.	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 CHI3L3 Conjugate	893840	12 mL of a polyclonal antibody specific for mouse CHI3L3 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse CHI3L3 Standard	893841	20 ng of recombinant mouse CHI3L3 in a buffered protein base with preservatives; lyophilized.	
Mouse CHI3L3 Control	893842	Recombinant mouse CHI3L3 in a buffered protein base with preservatives; lyophilized. The concentration range of mouse CHI3L3 after reconstitution is shown on the vial label. 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 RD5-26 Concentrate	895525	21 mL of a concentrated 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.
- 100 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.

If using cell lysate samples, the following is also required:

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

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.

Cell Lysates - Cell must be lysed prior to assay as directed 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.

Plasma - Collect plasma using EDTA or heparin 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: *Citrate plasma has not been validated for use in this assay.*
Do not use hemolyzed or icteric samples.

SAMPLE PREPARATION

Serum and plasma samples require a 50-fold dilution. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5-26 (1X).

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse CHI3L3 Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

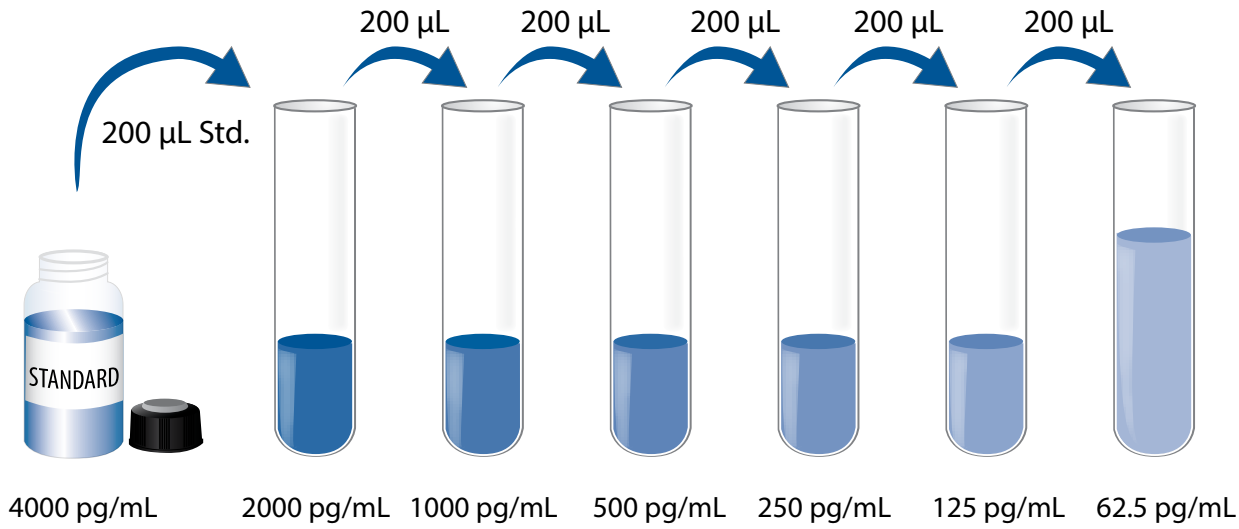
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD5-26 (1X) - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (1X).

Mouse CHI3L3 Standard - Reconstitute the Mouse CHI3L3 Standard with 5.0 mL of Calibrator Diluent RD5-26 (1X). This reconstitution produces a stock solution of 4000 pg/mL. Mix the standard to ensure complete reconstitution, and allow the standard to sit for a minimum 5 minutes with gentle agitation prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-26 (1X) into six tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse CHI3L3 Standard (4000 pg/mL) serves as the high standard. Calibrator Diluent RD5-26 (1X) 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 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 CHI3L3 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. The color in the wells should change from blue to yellow.
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 dilution. See Sample Preparation section.

CALCULATION OF RESULTS

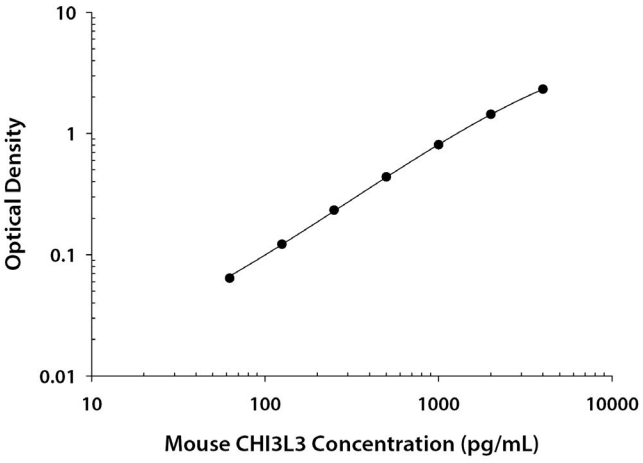
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

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 CHI3L3 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, 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	0.014 0.014	0.014	—
62.5	0.077 0.079	0.078	0.064
125	0.135 0.136	0.136	0.122
250	0.242 0.251	0.247	0.233
500	0.439 0.464	0.452	0.438
1000	0.797 0.842	0.820	0.806
2000	1.408 1.495	1.452	1.438
4000	2.315 2.355	2.335	2.321

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 forty 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	40	40	40
Mean (pg/mL)	90.6	451	812	97.4	432	797
Standard deviation	4.8	16.8	16.0	8.6	28.1	34.8
CV (%)	5.3	3.7	2.0	8.8	6.5	4.4

RECOVERY

The recovery of mouse CHI3L3 spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=8)	96	88-101%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse CHI3L3 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture samples (n=4)	Cell lysates (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	101	102	102	103	101
	Range (%)	97-106	99-105	98-105	100-106	95-105
1:4	Average % of Expected	101	101	98	100	98
	Range (%)	98-105	95-106	93-104	96-104	88-107
1:8	Average % of Expected	100	105	96	102	95
	Range (%)	96-108	100-111	92-100	102-102	85-107
1:16	Average % of Expected	98	104	95	99	95
	Range (%)	95-103	96-115	91-99	99-99	85-109

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

SENSITIVITY

Twenty-seven assays were evaluated and the minimum detectable dose (MDD) of mouse CHI3L3 ranged from 1.8-10.6 pg/mL. The mean MDD was 3.5 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 NS0-expressed recombinant mouse CHI3L3 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse CHI3L3 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=20)	67.3	16.5-189	61.1
EDTA plasma (n=20)	24.7	5.9-73	19.6
Heparin plasma (n=20)	55.1	6.6-188	50.6

Cell Culture Supernates - Organs from 2-3 mice were removed and pooled. Small slits were cut into the hearts to allow excess blood to drain. The organs were then rinsed with PBS, placed into tubes containing PBS, and stored on ice. Organs were chopped into 1-2 mm pieces and cultured in 100 mL of RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were unstimulated or stimulated with 1 µg/mL of lipopolysaccharide for 4 days. Aliquots the cell culture supernates were removed and assayed for levels of natural mouse CHI3L3.

Tissue Type	Observed Levels (ng/mL)
Brain	ND
Heart	ND
Kidney, Unstimulated	0.224
Kidney, Stimulated	0.223
Liver, Unstimulated	6.79
Liver, Stimulated	5.88
Lung, Unstimulated	2.81
Lung, Stimulated	2.63
Spleen, Unstimulated	15.3
Spleen, Stimulated	14.7

ND=Non-detecatble

SAMPLE VALUES *CONTINUED*

Cell Lysates - Organs from 2-3 mice were rinsed with PBS to remove excess blood, chopped into 1-2 mm pieces, homogenized with a tissue homogenizer, and 1 mL of Cell Lysis Buffer 2 was added (2 mL of Cell Lysis Buffer 2 was added to liver tissue). Organs were lysed at room temperature for 30 minutes with gentle agitation and centrifuged to remove debris. An aliquot of each cell lysate was removed and assayed for levels of natural mouse CHI3L3.

Tissue Type	Observed Levels (ng/mL)
Brain	3.57
Heart	17.0
Kidney	45.3
Liver	106
Lung	711
Spleen	2665

SPECIFICITY

This assay recognizes natural and recombinant mouse CHI3L3.

The factors listed below were prepared at 500 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 500 ng/mL in a mid-range recombinant mouse CHI3L3 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:
CHI3L1
CHIT1
TRANCE/RANK L

Recombinant human:
CHI3L1
CHI3L2
CHIT1

Other factors:
β-Collagen
Hyaluronan

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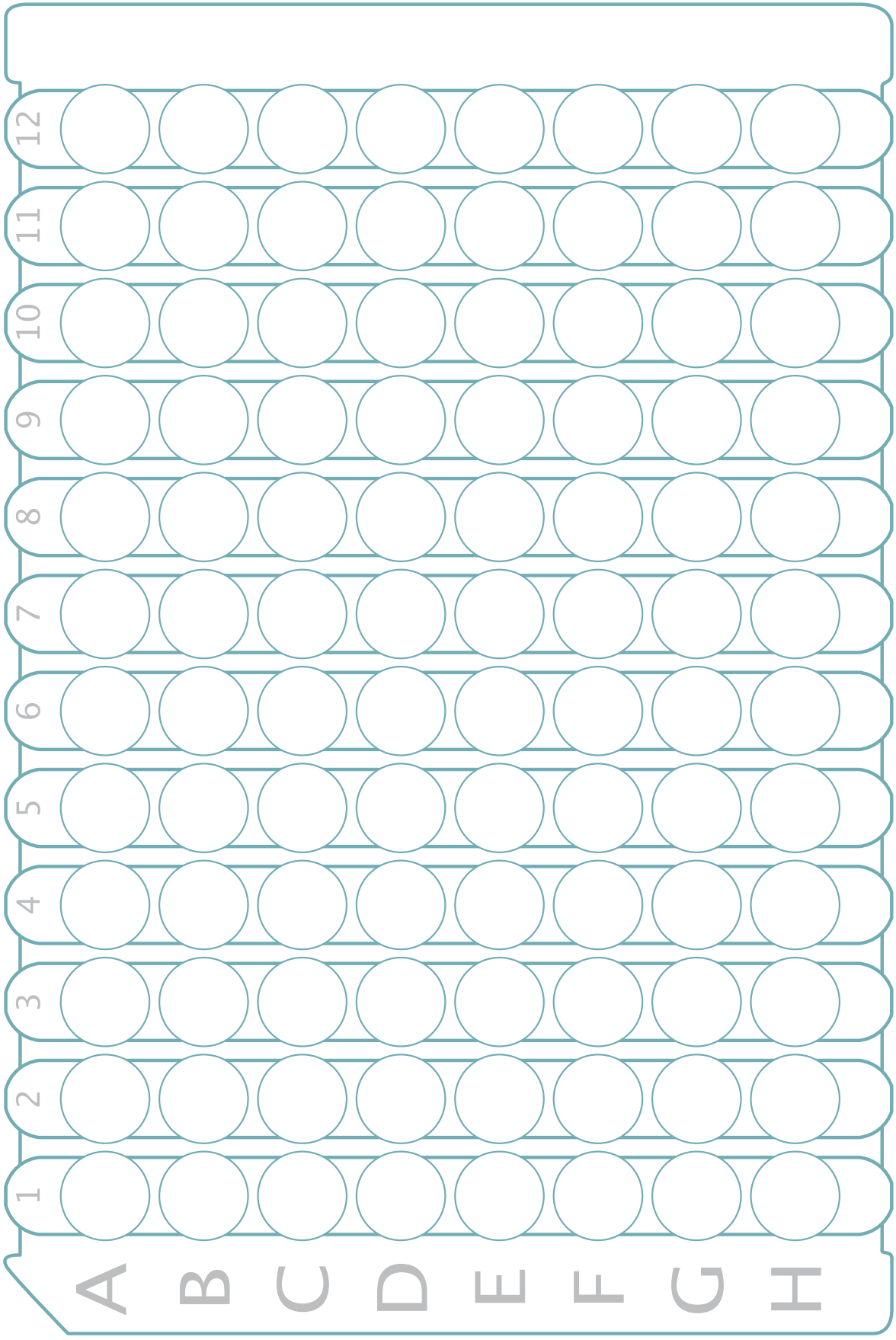
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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