Quantikine® ELISA

Mouse Clusterin Immunoassay

Catalog Number MCLU00

For the quantitative determination of mouse Clusterin concentrations in cell culture supernates, cell lysates, serum, plasma, and urine.

Note: The standard reconstitution method has changed. 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

Clusterin, also known as Apolipoprotein J, Sulfated Glycoprotein 2 (SGP-2), TRPM-2, and SP-40, is a secreted multifunctional protein that was named for its ability to induce cellular clustering. It binds a wide range of molecules and may function as a chaperone of misfolded extracellular proteins. It also participates in the control of cell proliferation, apoptosis, and carcinogenesis (1, 2). Clusterin is expressed in adult testis, ovary, adrenal gland, liver, heart, brain, and in many epithelial tissues during embryonic development (3). Mouse Clusterin is synthesized as a precursor that contains two coiled coil domains, two nuclear localization signals (NLS), and one heparin binding domain (4-6). Intracellular cleavages of the precursor remove the signal peptide and generate comparably sized α and β chains which are secreted as an approximately 80 kDa N-glycosylated and disulfide-linked heterodimer (7-9). Mature mouse Clusterin shares 77% and 93% amino acid sequence identity with human and rat Clusterin, respectively.

An alternately spliced 50 kDa isoform of mouse Clusterin (nCLU) remains intracellular and is neither glycosylated nor cleaved into α and β chains (4). Cellular exposure to ionizing radiation promotes the translocation of nCLU to the nucleus where it interacts with Ku70 and promotes apoptosis (4, 10). This function contrasts with the cytoprotective effect of secreted Clusterin (11). High µg/mL concentrations of Clusterin circulate predominantly as a component of high density lipoprotein particles, and these are internalized and degraded through interactions with LRP-2/Megalin (12, 13). The ability of Clusterin to bind and neutralize non-oxidatively modified LDL reduces cytotoxicity in atherosclerotic plaques (14). The chaperone function of Clusterin also helps to reduce the accumulation of β-amyloid fibrils and damage due to amyloid plaques in Alzheimer's disease (15). Clusterin levels are elevated in the cerebrospinal fluid of patients with Alzheimer's disease, Parkinson's disease, and multiple sclerosis (9, 16, 17) and in the urine of patients with kidney injury or bladder cancer (18-20). Clusterin is released by activated platelets at sites of vascular injury and is found in the synovial fluid of rheumatoid arthritis and osteoarthritis patients (21, 22). During human tumor progression, nCLU is downregulated while the secreted form is upregulated and may be aberrantly glycosylated (10, 23-25). Increased circulating levels of Clusterin enhance tumor aggressiveness by inhibiting apoptosis and by promoting epithelial to mesenchymal transition (26-28).

The Quantikine® Mouse Clusterin Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse Clusterin in cell culture supernates, cell lysates, serum, plasma, and urine. It contains NS0-expressed recombinant mouse Clusterin and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant factor accurately. Results obtained using natural mouse Clusterin showed dose-response 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 mouse Clusterin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse Clusterin has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any Clusterin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse Clusterin 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 Clusterin 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.
- 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 Clusterin Microplate	894017	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse Clusterin.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse Clusterin Standard	894019	Recombinant mouse Clusterin in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store at ≤ -20 °C for up to 1 month.* Avoid repeated freeze-thaw cycles.
Mouse Clusterin Control	894020	2 vials of recombinant mouse Clusterin in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	Discard after use. Use a fresh control for each assay.
Mouse Clusterin Conjugate	894018	12 mL of a polyclonal antibody specific for mouse Clusterin 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 solution with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:4 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	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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** 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 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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Cell Lysates - Cells must be lysed prior to assay. See 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.*
Icteric samples are not suitable for use in this assay.

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 and plasma samples require a 2000-fold dilution. A suggested 2000-fold dilution can be achieved by adding 10 µL of sample to 390 µL of Calibrator Diluent RD5-26 (diluted 1:4)*. Complete the 2000-fold dilution by adding 10 µL of the diluted sample to 490 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

Cell lysate samples require a 20-fold dilution. A suggested 20-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

Urine samples require a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse Clusterin Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted. Use within 30 minutes of reconstitution.

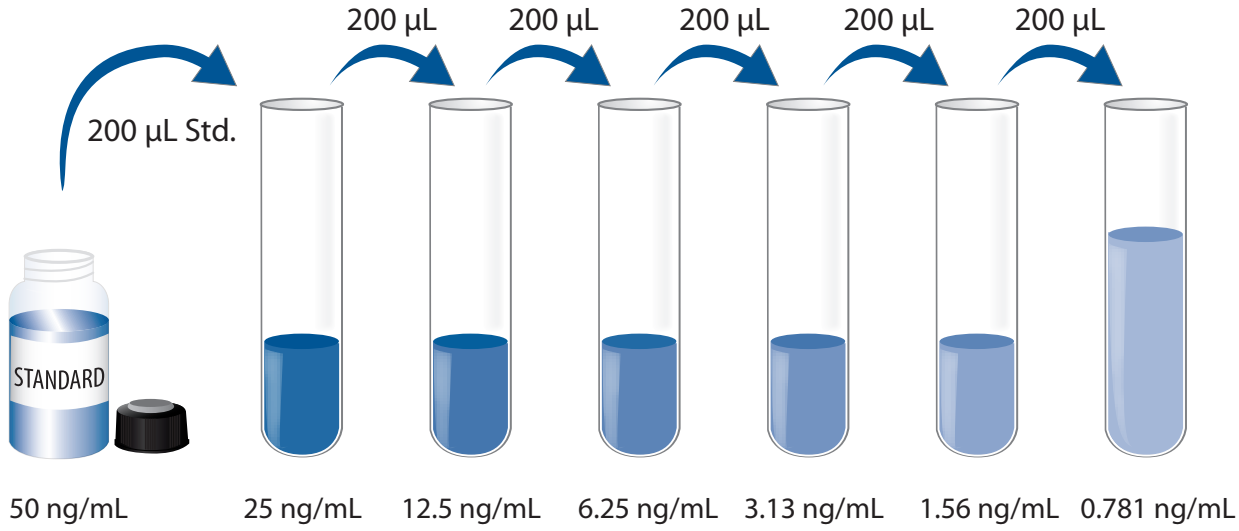
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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 (diluted 1:4) - 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 (diluted 1:4).

Mouse Clusterin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse Clusterin Standard with Calibrator Diluent RD5-26 (diluted 1:4). This reconstitution produces a stock solution of 50 ng/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 RD5-26 (diluted 1:4) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse Clusterin Standard (50 ng/mL) serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, control, and standards 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 on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. 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 Clusterin Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
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

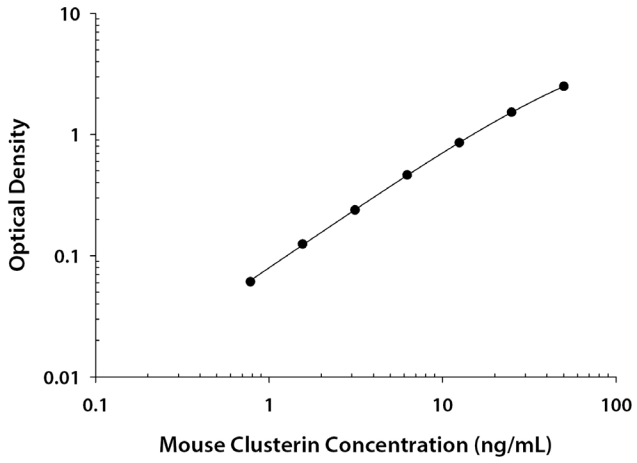
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 Clusterin 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.



(ng/mL)	O.D.	Average	Corrected
0	0.014 0.014	0.014	—
0.781	0.075 0.075	0.075	0.061
1.56	0.136 0.142	0.139	0.125
3.13	0.250 0.254	0.252	0.238
6.25	0.466 0.488	0.477	0.463
12.5	0.846 0.894	0.870	0.856
25	1.537 1.540	1.539	1.525
50	2.473 2.545	2.509	2.495

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 (ng/mL)	1.35	8.02	17.5	1.36	7.62	16.2
Standard deviation	0.086	0.282	0.682	0.119	0.577	1.62
CV (%)	6.4	3.5	3.9	8.8	7.6	10.0

RECOVERY

The recovery of mouse Clusterin spiked into various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	101	90-111%
Cell lysates (n=4)	106	95-117%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse Clusterin were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Cell lysates* (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine* (n=4)
1:2	Average % of Expected	90	99	97	96	94	95
	Range (%)	85-99	94-103	90-102	91-107	90-100	90-99
1:4	Average % of Expected	87	92	93	91	90	90
	Range (%)	83-95	87-95	91-96	86-102	86-96	84-98
1:8	Average % of Expected	92	87	89	89	88	89
	Range (%)	80-100	83-93	85-92	86-94	82-100	80-96
1:16	Average % of Expected	91	86	93	93	89	85
	Range (%)	91-91	81-94	83-97	87-96	80-102	80-93

*Samples were diluted prior to the assay.

SENSITIVITY

Sixty-six assays were evaluated and the minimum detectable dose (MDD) of mouse Clusterin ranged from 0.016-0.094 ng/mL. The mean MDD was 0.031 ng/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 NS0-expressed recombinant mouse Clusterin produced at R&D Systems®.

SAMPLE VALUES

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

Sample Type	Mean (µg/mL)	Range (µg/mL)	Standard Deviation (µg/mL)
Serum (n=20)	41.4	17.1-74.0	14.7
EDTA plasma (n=20)	48.1	23.0-72.4	11.8
Heparin plasma (n=20)	33.0	12.9-52.6	12.2

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Urine (n=10)	1802	814-3483	889

Cell Culture Supernates:
Organs from mice were removed, rinsed in 1X PBS, and kept on ice. The tissue was homogenized using a tissue homogenizer and seeded into media containing 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 for 1 or 3 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse Clusterin.

Tissue Type	(ng/mL)
Brain (1 day)	32.2
Heart (3 days)	8.29
Kidney (3 days)	29.6
Liver (3 days)	14.1
Lung (1 day)	41.3
Spleen (1 day)	12.0

L-929 mouse fibroblast cells (5 x 10^5 cells/mL) were cultured in MEM (NEAA) supplemented with 10% equine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were stimulated with 10 µg/mL PHA and 10 ng/mL PMA for 3 days. An aliquot of the cell culture supernate was removed, assayed for mouse Clusterin, and measured 3.43 ng/mL.

SAMPLE VALUES *CONTINUED*

Cell Lysates - Organs from mice were rinsed with 1X PBS and homogenized with a tissue homogenizer in 1X 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. An aliquot of each cell lysate was removed and assayed for levels of mouse Clusterin.

Tissue Type	(ng/mL)
Brain	618
Heart	254
Kidney	1034
Liver	171
Lung	1032
Spleen	389

SPECIFICITY

This assay recognizes natural and recombinant mouse Clusterin.

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

Recombinant mouse:
Apolipoprotein H

Recombinant human:
Apolipoprotein A-I
Apolipoprotein A-II
Apolipoprotein B
Apolipoprotein B100
Apolipoprotein C-I
Apolipoprotein D
Apolipoprotein E
Apolipoprotein M
Clusterin
Clusterin-like 1

Natural proteins:
Apolipoprotein A-I
Apolipoprotein A-II

Recombinant rat Clusterin cross-reacts approximately 0.5% in this assay.

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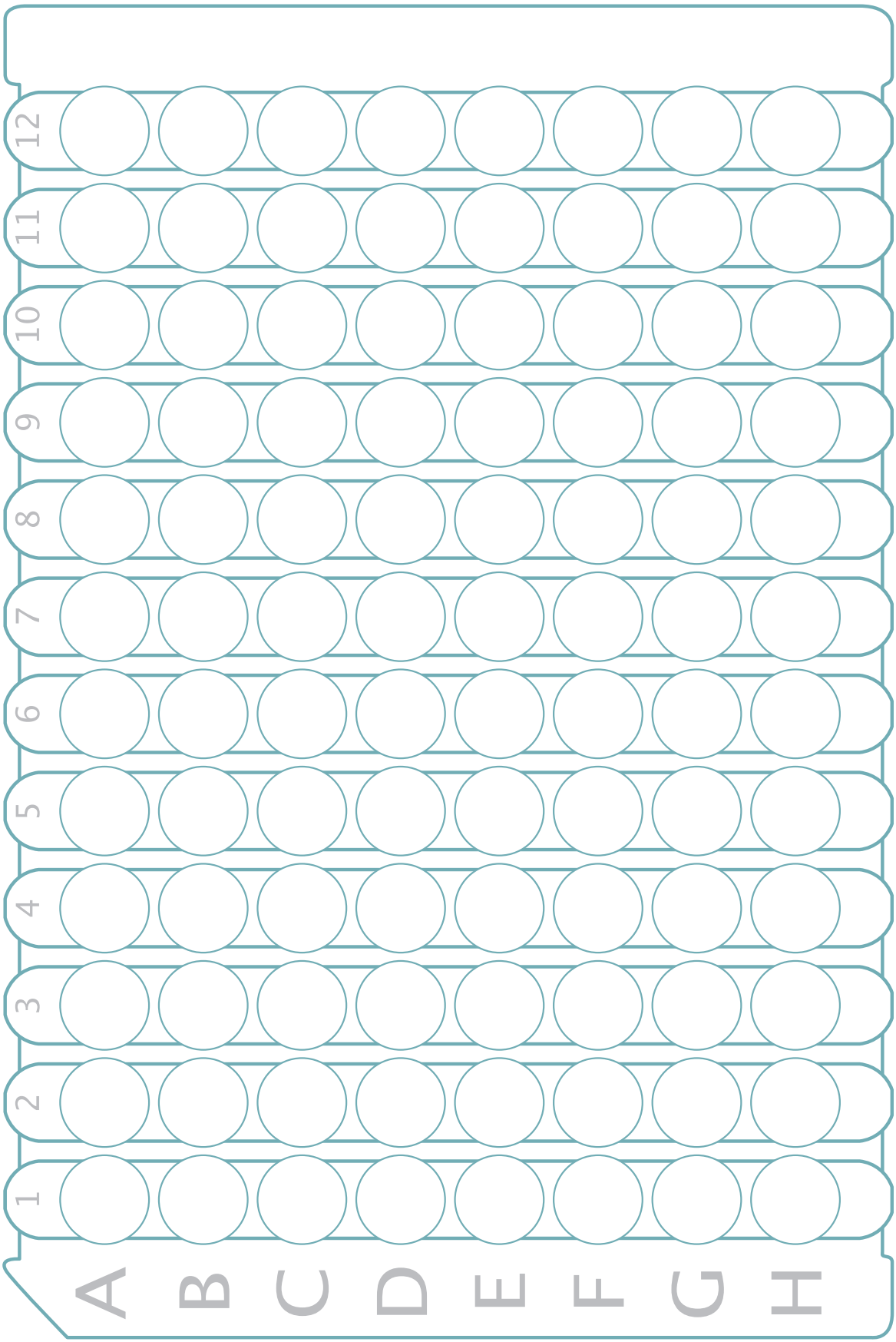
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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