Quantikine® ELISA

Mouse VCAM-1/CD106 Immunoassay

Catalog Number MVC00

For the quantitative determination of mouse Vascular Cell Adhesion Molecule-1 (VCAM-1) 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

Vascular cell adhesion molecule-1 (VCAM-1), also known as CD106, is a type I membrane glycoprotein belonging to the immunoglobulin superfamily (IgSF) of cell adhesion molecules (1-4). By alternative splicing, at least two variants of mouse VCAM-1 have been identified (1-8). The type I transmembrane mouse VCAM-1 long isoform is a 110 kDa protein containing a 674 amino acid (aa) residue extracellular domain organized into seven C2-type Ig domains, a 22 aa residue transmembrane domain and a 19 aa residue cytoplasmic domain (1-4). The short mouse VCAM-1 isoform is a 45 kDa glycosylphosphatidylinositol (GPI)-anchored protein containing only the first three amino-terminal Ig domains of the transmembrane isoform (1, 5-8). Both the transmembrane and the GPI-anchored VCAM-1 isoforms can be released from cells to yield soluble VCAM-1 that is detectable in cell supernates or serum (4, 6, 9). Various proteases, including MMPs, neutrophil elastase, and cathepsin B, have been implicated in the shedding of the transmembrane VCAM-1 (9, 10). The enzymes involved in the release of the GPI-anchored VCAM-1 are not known, but likely involve phospholipases (1, 4, 6). Mouse VCAM-1 has cross-species activity (2). The long isoform of mouse VCAM-1 shares 86% and 74% aa sequence identity to rat and human VCAM-1, respectively. The 19 aa residue cytoplasmic tail is identical in all three proteins (2, 4).

VCAM-1 is expressed on vascular endothelium upon induction by a number of inflammatory stimuli (4). It is also expressed constitutively on non-vascular cells, including dendritic cells in lymphoid tissues and skin (4), tissue macrophages (4), fibroblasts (11), smooth muscle cells (12), bone marrow stromal cells (13), liver Kupffer cells (14), chondrocytes (15), mesothelium (16), renal tubular epithelium and mesangium (17), embryonic myoblasts and myotubes (18), adult satellite muscle cells (18), melanoma cells (4), and choroid plexus epithelium (19).

VCAM-1 mediates cell adhesion and signal transduction by binding to its ligands/counter-receptors. The key VCAM-1 ligand expressed on all leukocytes is the integrin $\alpha_4\beta_1$/VLA-4 that binds to VCAM-1 at the first and fourth Ig domain (4, 20, 21). Additional VCAM-1 ligands include $\alpha_4\beta_7$ which is expressed on eosinophils, B and T cells (4, 22, 23), $\alpha_D\beta_2$ which is expressed on monocytes, macrophages, NK cells, eosinophils, basophils and neutrophils (24, 25), and $\alpha_9\beta_1$ which is expressed on neutrophils (26). Whereas $\alpha_4\beta_1$-mediated binding to VCAM-1 is constitutively active, the $\alpha_4\beta_7$-mediated binding of eosinophils to VCAM-1 requires activation of the integrin (22, 23, 27).

VCAM-1 is important for the recruitment of leukocytes to sites of inflammation and contributes to leukocyte extravasation (4). Constitutively expressed VCAM-1 on bone marrow endothelium and stromal cells regulates T and B cell development as well as hemopoietic progenitor cell homing and trafficking (28-30). VCAM-1 mediates the adhesion of melanoma cells to endothelial cells and may play a role in metastasis (4). VCAM-1 binding induces MMP-2 production in T cells, contributing to its migration through the extracellular matrix (4, 31). Soluble VCAM-1 has been shown to mediate angiogenesis and is chemotactic for T lymphocytes and monocytes (4, 32). The VCAM-1/integrin interaction may contribute to pathological conditions such as atherosclerosis, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, and allograft rejection (4).

The Quantikine Mouse VCAM-1/CD106 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse VCAM-1 levels in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant mouse VCAM-1 and antibodies raised against the recombinant protein. Results obtained for naturally occurring mouse VCAM-1 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 VCAM-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse VCAM-1 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse VCAM-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse VCAM-1 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 VCAM-1 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.

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 VCAM-1 Microplate	891098	96 well microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse VCAM-1.	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 VCAM-1 Standard	891100	2 vials of recombinant mouse VCAM-1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a new Standard and Control for each assay.
Mouse VCAM-1 Control	891101	2 vials of recombinant mouse VCAM-1 in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Mouse VCAM-1 Conjugate	891099	12 mL of a monoclonal antibody specific for mouse VCAM-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-21	895215	12 mL of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Assay Diluent RD1-47	895524	12 mL of a buffer solution with preservatives. *For serum/plasma samples*.	
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.
- **Polypropylene** 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 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.

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.*

SAMPLE PREPARATION

Use polypropylene tubes.

Serum and plasma samples require at least a 50-fold dilution prior to assay. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5-26 (diluted 1:4).*

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse VCAM-1 Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

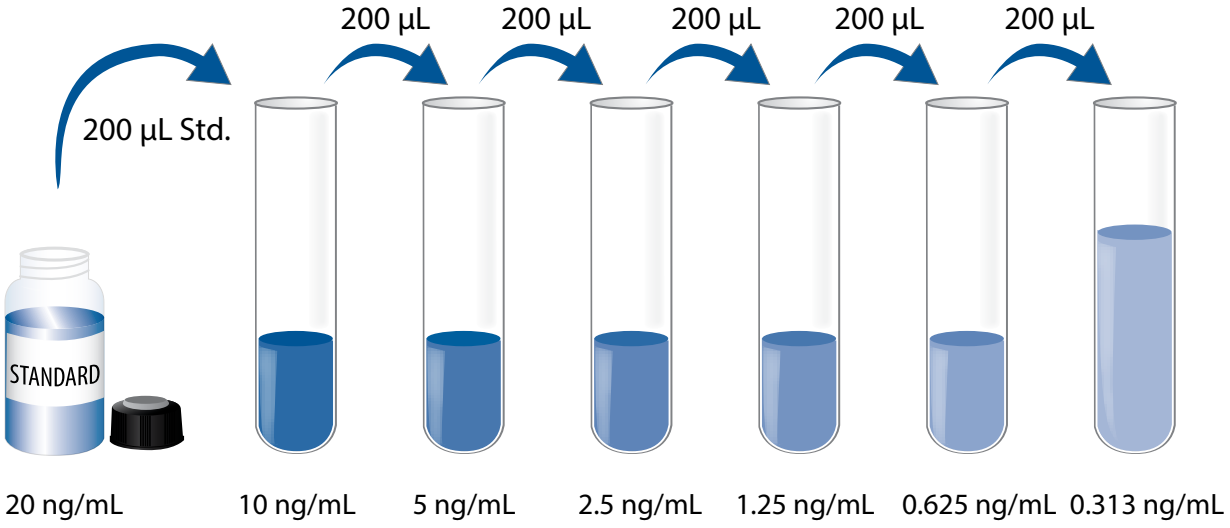
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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).

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 VCAM-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse VCAM-1 Standard with Calibrator Diluent RD5-26 (diluted 1:4). Do not substitute other diluents. This reconstitution produces a stock solution of 20 ng/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.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-26 (diluted 1:4) 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 VCAM-1 Standard (20 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 standards, Control, and samples be assayed in duplicate.

1. Prepare reagents, standards, 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 RD1-21 (*for cell culture supernate samples*) or Assay Diluent RD1-47 (*for serum/plasma samples*) 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 3 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 VCAM-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour 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.

*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.).

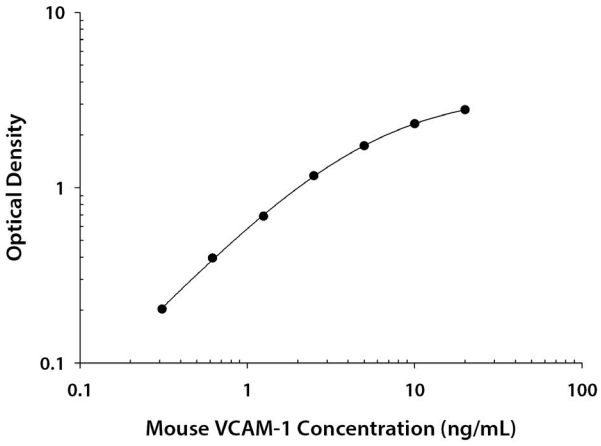
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse VCAM-1 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

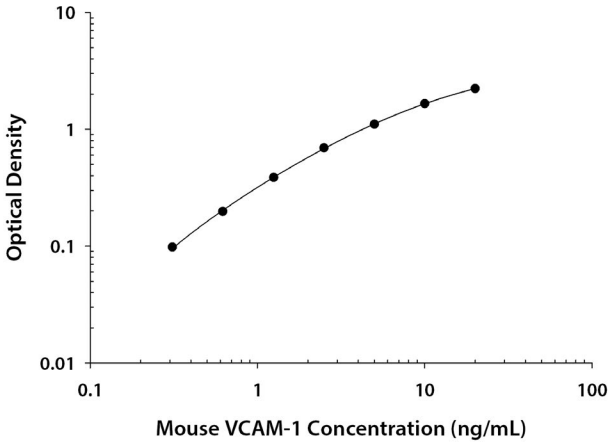
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.000 0.001	0.001	—
0.313	0.195 0.213	0.204	0.203
0.625	0.374 0.421	0.398	0.397
1.25	0.650 0.724	0.687	0.686
2.5	1.136 1.205	1.170	1.169
5	1.712 1.757	1.734	1.733
10	2.261 2.372	2.316	2.315
20	2.754 2.805	2.780	2.779

SERUM/PLASMA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.001 0.003	0.002	—
0.313	0.097 0.103	0.100	0.098
0.625	0.197 0.202	0.200	0.198
1.25	0.384 0.393	0.388	0.386
2.5	0.688 0.700	0.694	0.692
5	1.096 1.110	1.103	1.101
10	1.635 1.680	1.658	1.656
20	2.213 2.239	2.226	2.224

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.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	0.96	2.84	8.55	0.87	2.64	8.50
Standard deviation	0.05	0.13	0.37	0.07	0.18	0.63
CV (%)	5.2	4.6	4.3	8.0	6.8	7.4

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	0.93	2.61	8.58	0.86	2.46	8.46
Standard deviation	0.07	0.17	0.40	0.05	0.11	0.65
CV (%)	7.5	6.5	4.7	5.8	4.5	7.7

RECOVERY

The recovery of mouse VCAM-1 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernate (n=6)	100	89-112%
Serum* (n=10)	99	80-118%
EDTA plasma* (n=4)	100	81-119%

*Samples were diluted prior to assay.

SENSITIVITY

Four assays were evaluated and the minimum detectable dose (MDD) of mouse VCAM-1 ranged from 20-60 pg/mL. The mean MDD was 30 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.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse VCAM-1 were diluted with Calibrator Diluent and assayed.

		Cell culture supernates (n=6)	Serum* (n=6)	EDTA plasma* (n=6)
1:2	Average % of Expected	106	103	101
	Range (%)	102-112	95-111	93-106
1:4	Average % of Expected	105	104	102
	Range (%)	101-115	96-113	97-109
1:8	Average % of Expected	105	106	100
	Range (%)	103-108	99-113	92-110
1:16	Average % of Expected	112	99	101
	Range (%)	108-116	95-107	92-112

*Samples were diluted prior to assay.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant mouse VCAM-1 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse VCAM-1 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Serum (n=22)	679	396-1275
EDTA plasma (n=19)	496	266-854

Cell Culture Supernates:
Lungs from mice were cultured for 5 days in 40 mL of RPMI supplemented with 10% fetal calf serum and stimulated with 10 µg/mL ConA. An aliquot of the cell culture supernate was removed, assayed for mouse VCAM-1, and measured 28.2 ng/mL.

Hearts from mice were cultured for 6 days in 40 mL of RPMI supplemented with 10% fetal calf serum. An aliquot of the cell culture supernate was removed, assayed for mouse VCAM-1, and measured 2.3 ng/mL.

L-929 mouse fibroblast cells (1 x 10^6 cells/mL) were cultured for 3 days in MEM supplemented with 10% equine serum and stimulated with 2.5 ng/mL LPS. An aliquot of the cell culture supernate was removed, assayed for mouse VCAM-1, and measured 1.5 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse VCAM-1.

The factors listed below were prepared at 50 ng/mL or 100 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL or 100 ng/mL in a mid-range mouse VCAM-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C10
Eotaxin
E-Selectin
Fas Ligand
Flt-3 Ligand
G-CSF
GM-CSF
ICAM-1
ICAM-2
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/IL-23 p40
IL-12 p70
IL-13
IL-17
IL-18
JE/MCP-1
KC
Leptin
LIF
L-Selectin
MARC
MCP-5
M-CSF
MIP-1α
MIP-1β
MIP-1γ
MIP-2
OPG
OSM
PlGF-2
P-Selectin
RANK
RANK Ligand
RANTES
SCF
TARC
TNF-α
TNF RI
TNF RII
Tpo
VEGF
VEGF RI

Recombinant human VCAM-1 cross-reacts approximately 6% in this assay.

Normal rat and porcine serum samples were evaluated and measured less than the lowest standard, 0.313 ng/mL.

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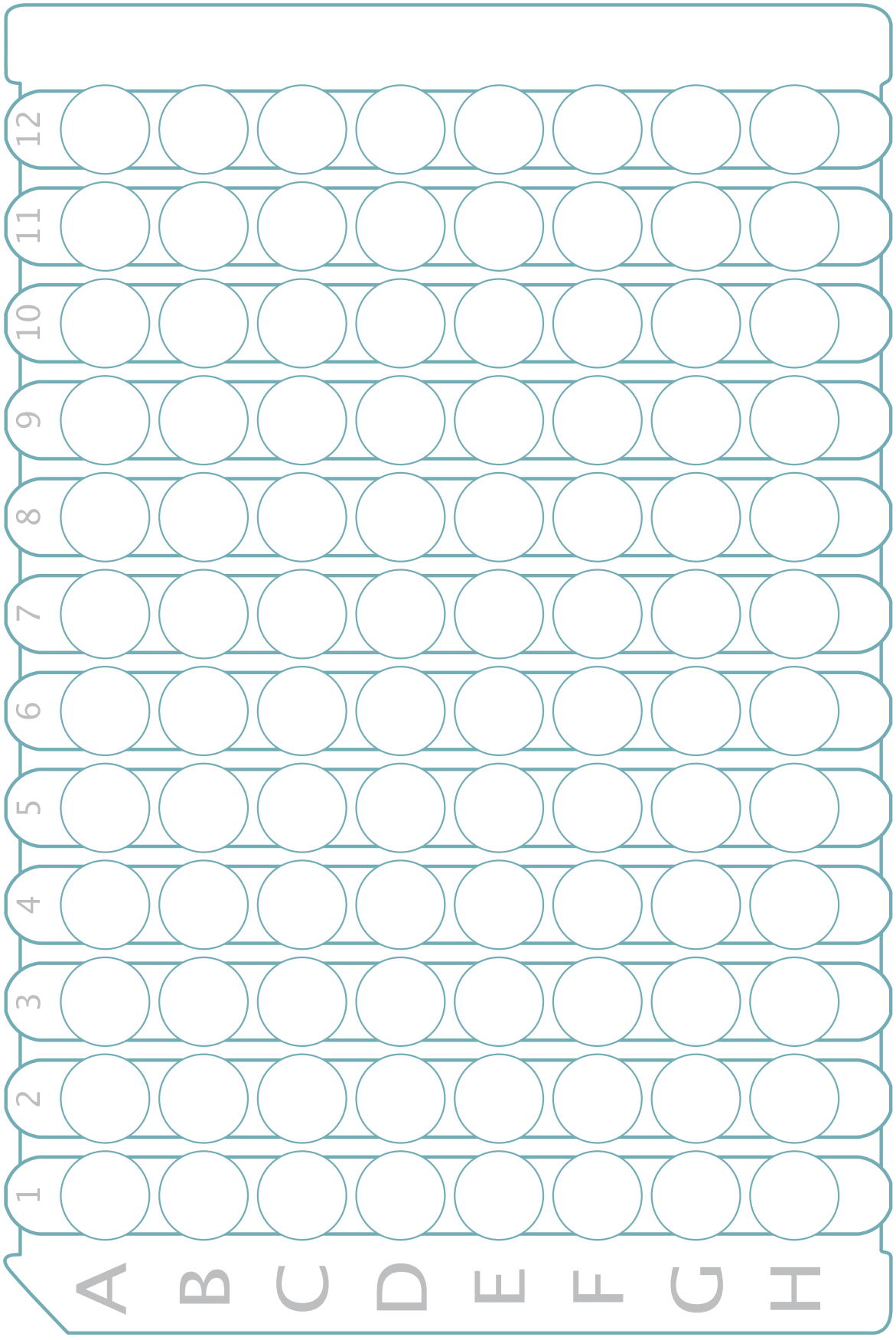
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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