Quantikine® ELISA

Mouse E-Selectin/CD62E Immunoassay

Catalog Number MES00

For the quantitative determination of mouse E-Selectin concentrations in cell culture supernates, 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

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INTRODUCTION

E-Selectin (also known as CD62E and ELAM-1), P-Selectin and L-Selectin are members of a small family of leukocyte adhesion molecules that share common structural motifs (1, 2). Mature mouse E-Selectin is an inducible, 117 kDa, type I transmembrane glycoprotein that is transiently and principally expressed on endothelial cells (EC) after activation by cytokines or bacterial endotoxin (3-5). Its extracellular region contains a 100 amino acid (aa) N-terminal C-type lectin domain, a 40 aa EGF-like motif, and six 60 aa short concensus repeats (SCRs, also known as Sushi domains). The molecule also has a 22 aa transmembrane segment and a short 33 aa cytoplasmic region that can transduce a signal in endothelial cells via mitogen-activated protein (MAP) kinase (6). E-Selectin from different species contains varying numbers of SCRs (7-9). Mouse E-Selectin shares 73%, 87%, and 67% aa identity to human, rat, and porcine E-Selectin, respectively (7-9). Mouse E-Selectin is active on human cells (5). Cells known to express E-Selectin include endothelial cells (10, 11), $CD4^+$ T cells (12), and renal tubular epithelial cells (13).

E-Selectin mediates the initial rolling and subsequent stable adhesion of leukocytes, and allows for leukocyte activation by chemokines, leading to their extravasation at sites of inflammation (1, 2, 14, 15). In general, E-Selectin binds sialyl Lewis-x (sLe^x) displayed on a number of glycoproteins or proteoglycans, including E-Selectin ligand (ESL) (16), P-Selectin glycoprotein ligand-1 (PSGL-1) (17), cutaneous lymphocyte antigen (CLA; a variant of PSGL-1) (18), L-Selectin (2) and β_2-integrin (19). The role for many of these potential E-Selectin ligands is not clearly understood. Integrin activation may involve E-Selectin, either indirectly as a consequence of E-Selectin binding to leukocyte E-Selectin ligand(s), or through direct integrin-E-Selectin interaction. The binding of E-Selectin to the leukocyte integrin CD11/CD18 can lead to integrin activation (19-26).

A 110 kDa soluble form of E-Selectin is known to exist and is assumed to arise from proteolytic cleavage (3, 5, 27, 28). Cell surface E-Selectin molecules are rapidly shed following EC activation. Soluble E-Selectin is reported to suppress leukocyte migration by competing with transmembrane E-Selectin. Soluble E-Selectin has been reported to be a mediator of angiogenesis and induce monocyte chemotaxis (29, 30).

The Quantikine® Mouse E-Selectin/CD62E Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse E-Selectin levels in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant mouse E-Selectin and antibodies raised against the recombinant protein. Results obtained using natural mouse E-Selectin 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 E-Selectin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse E-Selectin has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any E-Selectin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse E-Selectin 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 E-Selectin 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.
- It is recommended that the samples be pipetted within 10 minutes.
- 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 E-Selectin Microplate	890985	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse E-Selectin.	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 E-Selectin Conjugate	890986	12 mL of a monoclonal antibody specific for mouse E-Selectin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse E-Selectin Standard	890987	Recombinant mouse E-Selectin in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse E-Selectin Control	890988	Recombinant mouse E-Selectin 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 RD1-21	895215	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. *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

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. 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 E-Selectin Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

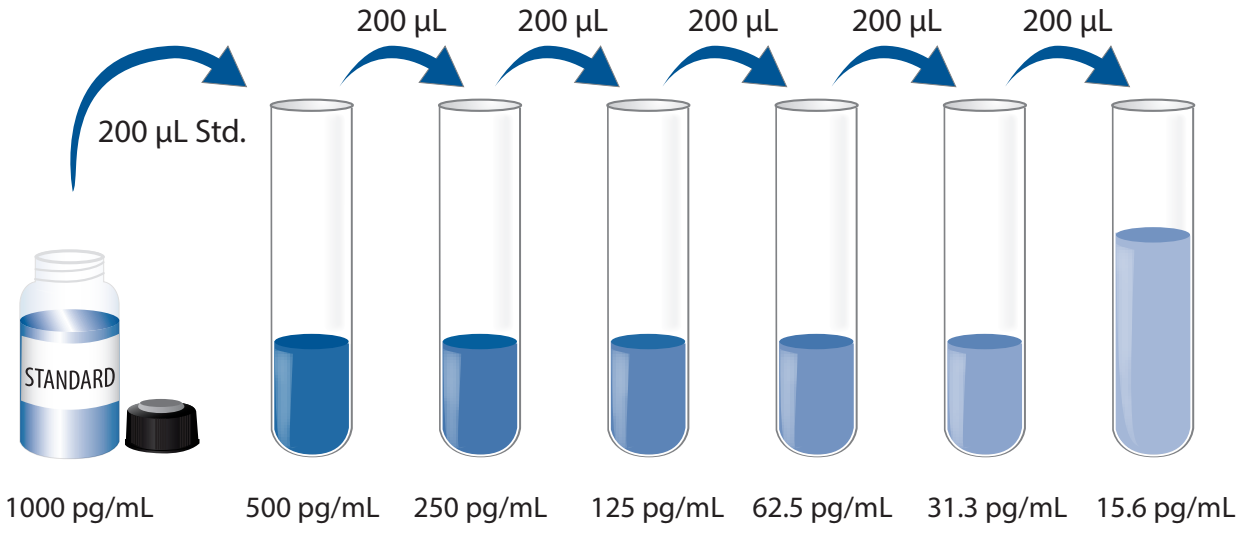
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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 (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 E-Selectin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse E-Selectin Standard with Calibrator Diluent RD5-26 (diluted 1:4). Do not substitute other diluents. This reconstitution produces a stock solution of 1000 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.

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 dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse E-Selectin Standard (1000 pg/mL) serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) 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 RD1-21 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 E-Selectin 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 mL 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

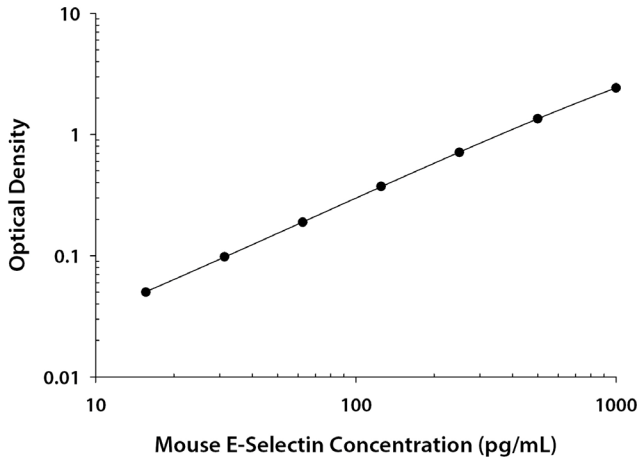
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 E-Selectin 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	0.025 0.021	0.023	—
15.6	0.069 0.077	0.073	0.050
31.3	0.121 0.121	0.121	0.098
62.5	0.212 0.212	0.212	0.189
125	0.392 0.401	0.396	0.373
250	0.728 0.745	0.736	0.713
500	1.371 1.373	1.372	1.349
1000	2.444 2.450	2.447	2.424

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 (pg/mL)	52	152	464	49	144	440
Standard deviation	4.6	9.5	41	4.3	10	29
CV (%)	8.8	6.3	8.8	8.8	6.9	6.6

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates (n=6)	95	83-102%
Serum* (n=10)	98	83-118%
EDTA plasma* (n=4)	101	78-120%

*Samples were spiked and then diluted 100-fold prior to assay.

LINEARITY

To assess the linearity of the assay, samples containing and/or spike with high concentrations of mouse E-Selectin were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=5)	Serum* (n=6)	EDTA plasma* (n=6)
1:2	Average % of Expected	104	97	100
	Range (%)	98-109	89-101	91-112
1:4	Average % of Expected	105	98	104
	Range (%)	96-111	91-104	96-112
1:8	Average % of Expected	107	96	103
	Range (%)	99-117	93-100	93-114
1:16	Average % of Expected	110	98	106
	Range (%)	105-117	96-99	99-109

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

SENSITIVITY

Two assays were evaluated and the minimum detectable dose (MDD) of mouse E-Selectin ranged from 2.8-4.7 pg/mL. The mean MDD was 3.8 pg/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 E-Selectin produced at R&D Systems®.

SAMPLE VALUES

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

Sample Type	Mean (ng/mL)	Range (ng/mL)
Serum (n=20)	38	21-53
EDTA plasma (n=20)	26	18-52

Note: Rat and porcine serum samples were also evaluated for E-Selectin and measured less than the lowest standard, 15.6 pg/mL.

Cell Culture Supernates:
Mouse lung conditioned media (lungs from two mice) was cultured for 6 days in 40 mL RPMI supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for mouse E-Selectin, and measured 0.035 ng/mL.

WEHI-3 mouse myelomonocytic leukemia cells were cultured for 3 days in RPMI supplemented with 10% fetal bovine serum. Aliquots of the cell culture supernates were removed and assayed for mouse E-Selectin. No detectable levels were observed.

L929 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. Aliquots of the cell culture supernates were removed and assayed for mouse E-Selectin. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant mouse E-Selectin.

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

Recombinant mouse:

C10
Eotaxin
Fas Ligand
Flt-3 Ligand
G-CSF
GM-CSF
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
RANTES
SCF
TARC
TNF-α
TNF RI
TNF RII
Tpo
TRANCE
VCAM-1
VEGF
VEGF RI

Recombinant rat:

E-Selectin

Recombinant human E-Selectin cross-reacts approximately 2% in this assay.

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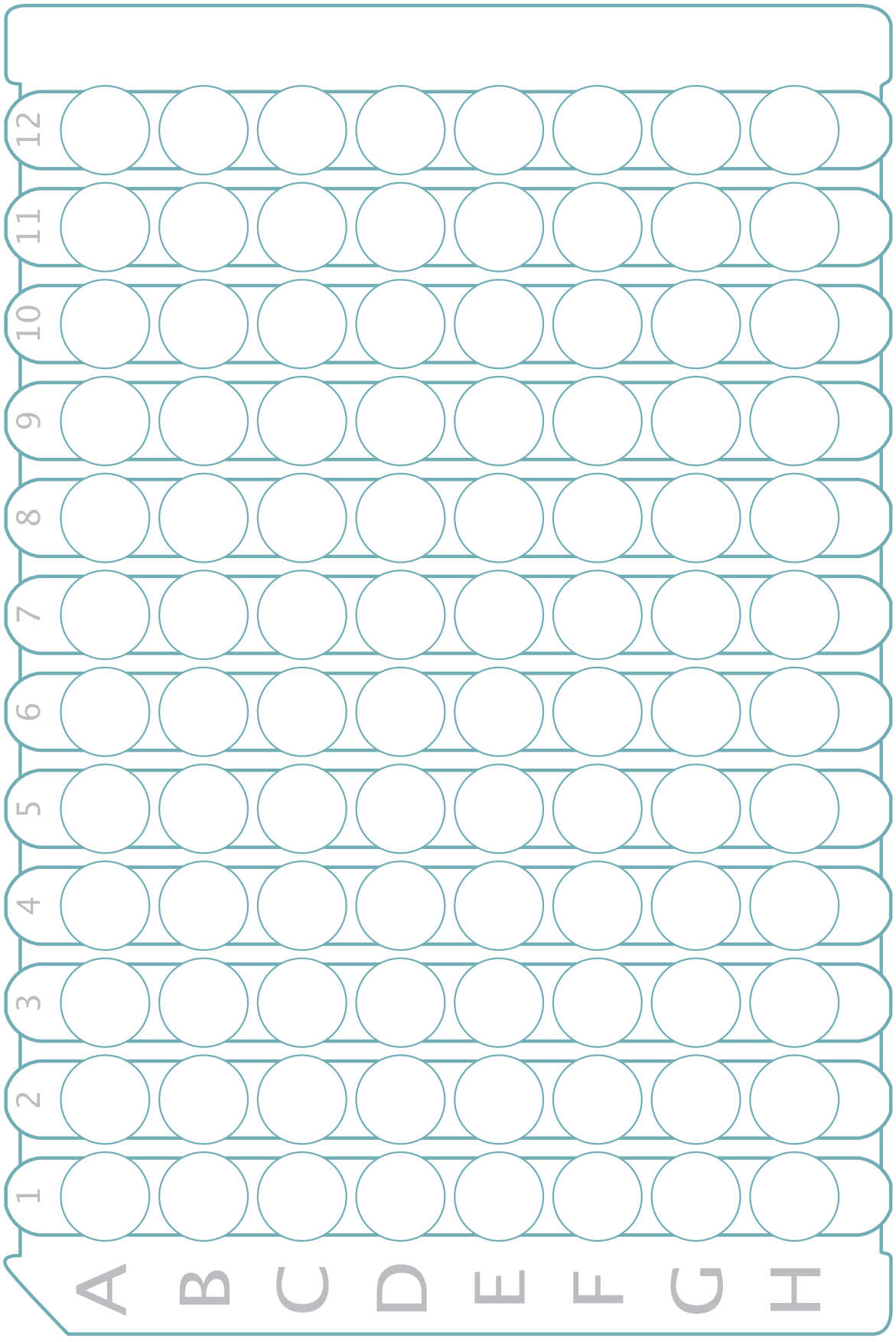
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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