Quantikine® ELISA

Human VE-Cadherin Immunoassay

Catalog Number DCADV0

For the quantitative determination of human VE-Cadherin 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.

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INTRODUCTION

The Cadherin (Ca^{++}-dependent adherence) superfamily is a large group of membrane-associated glycoproteins that engage in homotypic cell-cell adhesion events. VE-Cadherin, also known as Cadherin-5 and CD144, is a member of the atypical/type II subgroup of Cadherins (1, 2). Mature human VE-Cadherin is a 125-135 kDa type I transmembrane glycoprotein that consists of a 552 amino acid (aa) extracellular domain (ECD) with five Ca^{++}-binding Cadherin domains, a 21 aa transmembrane segment, and a 164 aa cytoplasmic domain (3, 4). Within the ECD, human VE-Cadherin shares approximately 75% aa sequence identity with mouse and rat VE-Cadherin. A 90 kDa portion of the VE-Cadherin ECD is shed in a metalloproteinase-dependent mechanism (5). VE-Cadherin is expressed on the surface of vascular endothelial cells (EC) from early in embryogenesis through adulthood as well as in the placenta and on hematopoietic cell progenitors (6, 7). It is a major protein component of adherens junctions between cells of the endothelium (5, 8). The critical role of VE-Cadherin during embryogenesis is shown by widespread and lethal defects of vascular morphogenesis in mice that lack VE-Cadherin expression (9, 10). VE-Cadherin is not required for the adhesion between EC or the initiation of vascular sprouts (9, 11). Rather, it supports the extension and stabilization of existing vascular sprouts (12). VE-Cadherin is temporarily displaced from adherens junctions during leukocyte transmigration across the endothelium (13).

VE-Cadherin function is regulated in part by VEGF R2, a transmembrane receptor for the VEGF angiogenic factors. VEGF-induced signaling through VEGF R2 leads to increased EC detachment and trans-endothelial permeability by promoting VE-Cadherin internalization (14). In return, VE-Cadherin limits the proliferation of EC by preventing VEGF R2 internalization and signaling (15). VE-Cadherin also regulates or is regulated by other endothelial junction proteins such as JAM-C, Claudin-5, and N-Cadherin (16-18). VE-Cadherin, along with CD31/PECAM-1 and αV Integrins, mediates the EC response to shear stress (19). VE-Cadherin also promotes the association of type I and type II TGF-β receptors and is required for TGF-β receptor signaling in the endothelium (20).

VE-Cadherin contributes to breast cancer aggressiveness by promoting the adhesion of tumor cells to the vascular endothelium as well as by enhancing TGF-β induced signaling (21). Its blockade prevents *in vivo* angiogenesis and tumor growth (22, 23). Elevated levels of soluble VE-Cadherin are found in the serum of myocardial infarction, angina pectoris, and colorectal cancer patients (24, 25). This protein may function as a VE-Cadherin antagonist as suggested by the ability of a three-Cadherin-domain-molecule to inhibit *in vivo* tumor angiogenesis and tumor growth (26).

The Quantikine® Human VE-Cadherin Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human VE-Cadherin in cell culture supernates, serum, and plasma. It contains NS0-derived recombinant human VE-Cadherin and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human VE-Cadherin 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 natural human VE-Cadherin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human VE-Cadherin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any VE-Cadherin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human VE-Cadherin 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 VE-Cadherin 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 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.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate 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
Human VE-Cadherin Microplate	893798	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human VE-Cadherin.	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.*
Human VE-Cadherin Conjugate	893799	21 mL of a polyclonal antibody specific for human VE-Cadherin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human VE-Cadherin Standard	893800	Recombinant human VE-Cadherin in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-57	895207	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:5 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	895032	6 mL of 2 N sulfuric 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.
- Human VE-Cadherin Controls (optional; R&D Systems®, Catalog # QC184).

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 and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 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, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Cell culture supernate samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD5P (diluted 1:5)*.

Serum and plasma samples require a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5P (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

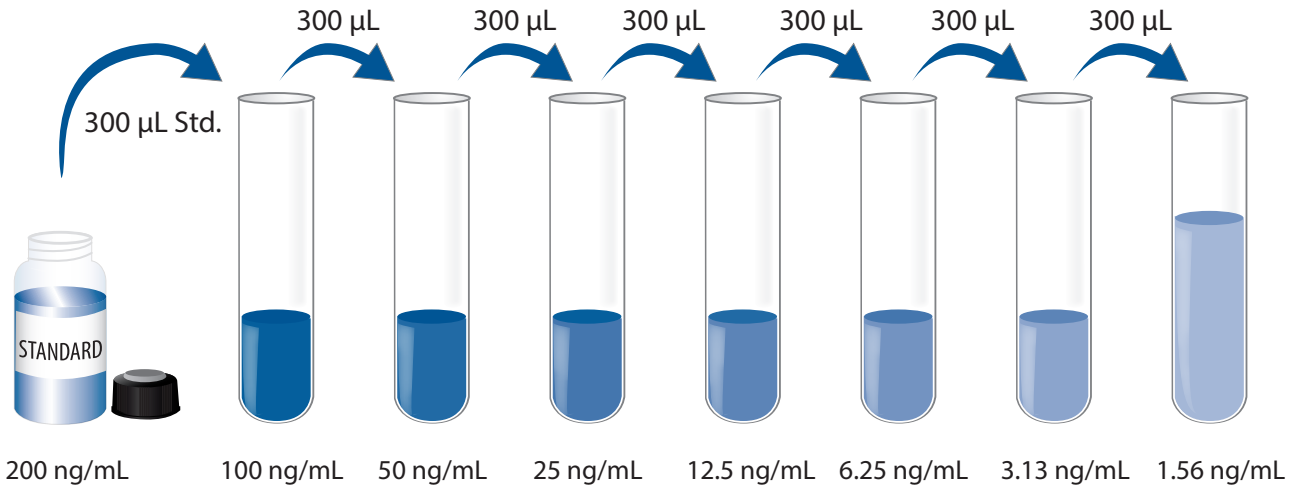
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Human VE-Cadherin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human VE-Cadherin Standard with deionized or distilled water. This reconstitution produces a stock solution of 200 ng/mL. Mix the standard to ensure complete reconstitution, and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 300 µL of Calibrator Diluent RD5P (diluted 1:5) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 100 ng/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) 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, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, 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 100 µL of Assay Diluent RD1-57 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 three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or auto washer. 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 200 µL of Human VE-Cadherin 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 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, 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 require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

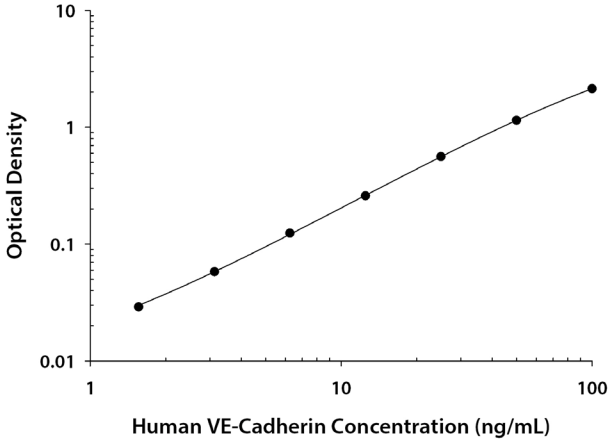
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human VE-Cadherin concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

Since 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	0.013 0.013	0.013	—
1.56	0.041 0.042	0.042	0.029
3.13	0.069 0.073	0.071	0.058
6.25	0.136 0.137	0.137	0.124
12.5	0.271 0.273	0.272	0.259
25	0.570 0.575	0.573	0.560
50	1.145 1.172	1.159	1.146
100	2.111 2.183	2.147	2.134

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 components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	13.4	34.6	65.4	12.8	31.8	64.5
Standard deviation	0.68	1.63	2.05	0.92	1.64	2.86
CV (%)	5.1	4.7	3.1	7.2	5.2	4.4

RECOVERY

The recovery of human VE-Cadherin spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	101	94-108%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human VE-Cadherin were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to this assay.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Citrate plasma (n=4)
1:2	Average % of Expected	106	105	105	105	104
	Range (%)	105-107	103-109	99-108	100-108	102-108
1:4	Average % of Expected	107	106	108	106	105
	Range (%)	106-109	103-111	103-116	104-108	103-106
1:8	Average % of Expected	110	109	110	107	106
	Range (%)	108-113	107-111	103-117	103-110	105-107
1:16	Average % of Expected	110	108	111	107	101
	Range (%)	105-112	104-111	103-119	105-108	99-102

SENSITIVITY

Forty-four assays were evaluated and the minimum detectable dose (MDD) of human VE-Cadherin ranged from 0.046-0.236 ng/mL. The mean MDD was 0.113 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 human VE-Cadherin produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human VE-Cadherin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=36)	2981	1880-4730	660
EDTA plasma (n=36)	2609	1375-4294	603
Heparin plasma (n=36)	2858	1965-4827	652
Citrate plasma (n=32)	2217	1380-3411	469

Cell Culture Supernates:
Human peripheral blood lymphocytes were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1 and 6 days. Aliquots of the cell culture supernates were removed and assayed for levels of human VE-Cadherin.

Condition	Day 1 (ng/mL)	Day 6 (ng/mL)
Unstimulated	5.36	4.54
Stimulated	4.86	4.38

SAMPLE VALUES *CONTINUED*

HUVEC human umbilical vein endothelial cells were cultured in EGM-2 growth media. Cells were cultured unstimulated or stimulated with 40 ng/mL recombinant human TNF-α and 50 ng/mL recombinant human IFN-γ or 10 ng/mL recombinant human IL-1β. Aliquots of the cell culture supernates were removed and assayed for levels of human VE-Cadherin.

Condition	Value (ng/mL)
Unstimulated	108
Stimulated w/TNF-α and IFN-γ	91
Stimulated w/IL-1β	81

HMVEC human microvascular endothelial cells were cultured in EGM-2 growth media. An aliquot of the cell culture supernate was removed, assayed for human VE-Cadherin, and measured 12.5 ng/mL.

JAR human choriocarcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum and 2 mM L-glutamine. An aliquot of the cell culture supernate was removed, assayed for human VE-Cadherin, and measured 7.12 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human VE-Cadherin.

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 human VE-Cadherin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ALCAM
ALK-1
BCAM
Cadherin-8
Cadherin-11
CD31/PECAM-1
CHL-1/L1CAM-2
Contactin-1
Contactin-2/TAG1
Contactin-4
DC-SIGNR/CD299
Desmoglein-1
Desmoglein-2
DNAM-1
E-Cadherin
Endocan
Endoglin/CD105
EpCAM/TROP1
E-Selectin
Galectin-1
Galectin-2
Galectin-3
Galectin-4
Galectin-7
Galectin-8
Galectin-9
ICAM-1
ICAM-2
ICAM-3
ICAM-5
JAM-A
JAM-B/VE-JAM
JAM-C
LOX-1/OLR1
L-Selectin
MCAM/CD146
M-Cadherin
N-Cadherin
NCAM-L1
NrCAM
P-Cadherin
P-Selectin
Siglec-2/CD22
Siglec-3/CD33
Siglec-5
Siglec-6
Siglec-7
Siglec-9
Siglec-10
TGF-β1
TGF-β2
TGF-β3
TGF-β RI/ALK-5
TGF-β RII
TGF-β RIIb
TGF-β RIII
TROP-2
VCAM-1

Recombinant mouse:

ALCAM
CHL-1/L1CAM-2
E-Cadherin
Endocan
E-Selectin
Galectin-1
Galectin-3
Galectin-7
ICAM-1
ICAM-2
ICAM-5
JAM-A
JAM-B/VE-JAM
JAM-C
LOX-1/OLR1
L-Selectin
MAdCAM-1
P-Cadherin
P-Selectin
Siglec-F
Siglec-2/CD22
VCAM-1

Recombinant rat:

E-Selectin
ICAM-1
L-Selectin
MAG/Siglec-4

Recombinant mouse VE-Cadherin cross-reacts approximately 3.7% in this assay.

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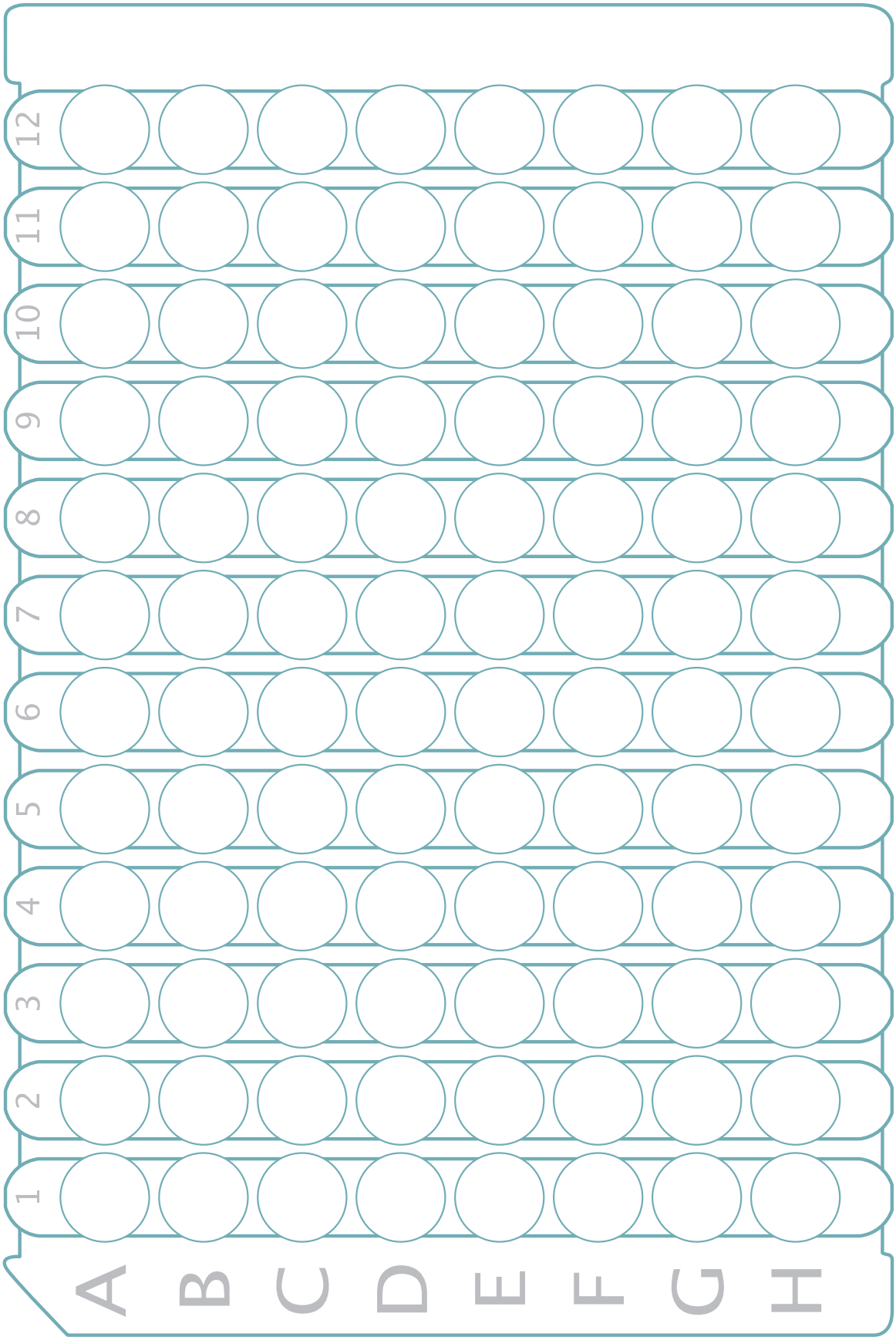
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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