Quantikine® ELISA

Human E-Cadherin Immunoassay

Catalog Number DCADE0

For the quantitative determination of human Epithelial Cadherin (E-Cadherin) concentrations in cell culture supernates, serum, plasma, urine, and saliva.

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

Epithelial Cadherin (E-Cadherin), also known as ECAD, Cadherin-1 (CDH1), Cell-CAM120/80, and Uvomorulin, is considered the prototypical member of the cadherin superfamily of cell adhesion molecules. E-Cadherin is a calcium (Ca^{2+})-dependent protein that binds in a homophilic manner and plays critical roles in epithelial cell-cell adhesion, morphogenesis, and maintenance of tissue architecture. Mature E-Cadherin, a type I classic cadherin, is a 120 kDa membrane-spanning glycoprotein localized primarily to adherens junctions (1, 2). The extracellular N-terminal domain consists of five repeated cadherin domains (C1-C5) of approximately 110 amino acids (aa) each (3). Ca^{2+} binding sequences including DAD, LDRE, PENE and DQND are found at the interdomain boundaries and are responsible for mediating Ca^{2+}-dependent adhesion (3). Upon Ca^{2+} binding, the extracellular domain converts from a globular structure to a rod-like, protease resistant conformation (4). E-Cadherin has a single-pass transmembrane domain and a short C-terminal cytoplasmic domain. The latter associates with multimolecular protein complexes that interact with the cytoskeleton, and regulates E-Cadherin activity and associated signal transduction. In addition to the transmembrane form, E-Cadherin is also found in a soluble form (sE-Cadherin), liberated from the cell surface by proteases including the matrix metalloproteases (MMPs) -3 and -7, and plasmin (5, 6).

Members of the intracellular domain protein complex regulating E-Cadherin function include the catenins. β- and γ-catenin, members of the Armadillo family, associate with the catenin-binding domain in the cytoplasmic tail of E-Cadherin. Another catenin, α-catenin, is shown to mediate interactions between the E-Cadherin/β- or γ-catenin complex and the F-actin cytoskeleton directly, or through direct and indirect interactions with several actin-binding proteins including vinculin, afadin, ZO-1, α-actinin, and the zyxin family (7-10).

Several mechanisms regulate E-Cadherin adhesive strength. For instance, the association between the E-Cadherin/β-catenin complex and α-catenin is inhibited by the protein IQGAP1, an effector of Rho family members Rac1 and Cdc42 (11). Phosphorylation of β-catenin has been shown to reduce its binding to E-Cadherin, also resulting in decreases in adhesion (12). $p120^{ctn}$, another member of the Armadillo family, binds to the cytoplasmic tail of E-Cadherin at a site more proximal to the membrane than β-catenin (13). Binding of $p120^{ctn}$ to E-Cadherin has been shown to both positively and negatively effect adhesion depending on the context (14, 15).

Several lines of evidence suggest that E-Cadherin does more than simply mediate adhesive activity; it may also act as a signaling receptor. For instance, homophilic ligation of E-Cadherin results in the activation of Rac1 and PI 3-kinase (16). Additionally, a mutation in the cytoplasmic tail of E-Cadherin that inhibits $p120^{ctn}$ association, blocks Rac1 activation and the formation of adhesive contacts between E-Cadherin-transfected CHO cells (17). E-Cadherin activation can also lead to actin reorganization at nascent cell contacts via direct interaction with the Arp2/3 complex, a mediator of actin assembly (18).

INTRODUCTION *CONTINUED*

E-Cadherin also acts as a tumor suppressor. Alterations in E-Cadherin expression and activities play a direct role in tumor progression. Cancer cell dispersion and metastases depend on a loss of cell/cell adhesion and many *in vitro* studies suggest that tumor invasion is enhanced when E-Cadherin activities are modulated (19). In addition, decreased or absent E-Cadherin expression has been associated with many tumor types *in vivo* including pancreatic, gastric, colonic, esophogeal, and hepatocellular carcinomas (20-24). Other studies have shown that the soluble form of E-Cadherin can enhance tumor cell invasion *in vitro*, and sE-Cadherin levels in urine and serum have been found to be elevated in cancer patients (5, 6, 25, 26). E-Cadherin can also affect transformation independent of its role in cellular adhesion. In addition to its part in the E-Cadherin adhesion complex, β-catenin is also a component of the canonical Wnt signaling pathway, acting as a transcription factor via LEF/TCF DNA binding proteins. In this capacity, β-catenin is also a proto-oncogene. It has been shown that E-Cadherin can antagonize these nuclear signaling pathways by depleting the transcriptionally active pool of β-catenin (27).

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

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human E-Cadherin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any E-Cadherin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human E-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 E-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.
- Samples, controls, and standards must be pipetted within 15 minutes.
- 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.

PRECAUTIONS

E-Cadherin is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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 SDS on our website prior to use.

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 E-Cadherin Microplate	892167	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human E-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 E-Cadherin Standard	892169	Recombinant human E-Cadherin in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C* in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Human E-Cadherin Conjugate	892168	21 mL of a polyclonal antibody specific for human E-Cadherin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-78	895839	11 mL of a buffered protein base containing blue dye and preservatives. *May contain crystals; warm to room temperature and mix well before use.*	
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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Collection device for saliva samples that has no protein binding or filtering capabilities such as a Salivette® or equivalent.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human E-Cadherin Controls (optional; R&D Systems®, Catalog # QC78).

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 heparin 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.

Note: *EDTA plasma is not suitable for use in this assay due to its chelating properties. Citrate plasma has not been validated for use in this assay.*

Saliva - Collect saliva using a collection device such as a Salivette or equivalent. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Saliva collector must not have any protein binding or filtering capabilities.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Use polypropylene tubes.

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 20-fold dilution. A suggested 20-fold dilution is 20 µL of sample + 380 µL of Calibrator Diluent RD5P (diluted 1:5)*.

Saliva samples require a 5-fold dilution. A suggested 5-fold dilution is 50 µL of sample + 200 µL of Calibrator Diluent RD5P (diluted 1:5)*.

Urine 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)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *E-Cadherin is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

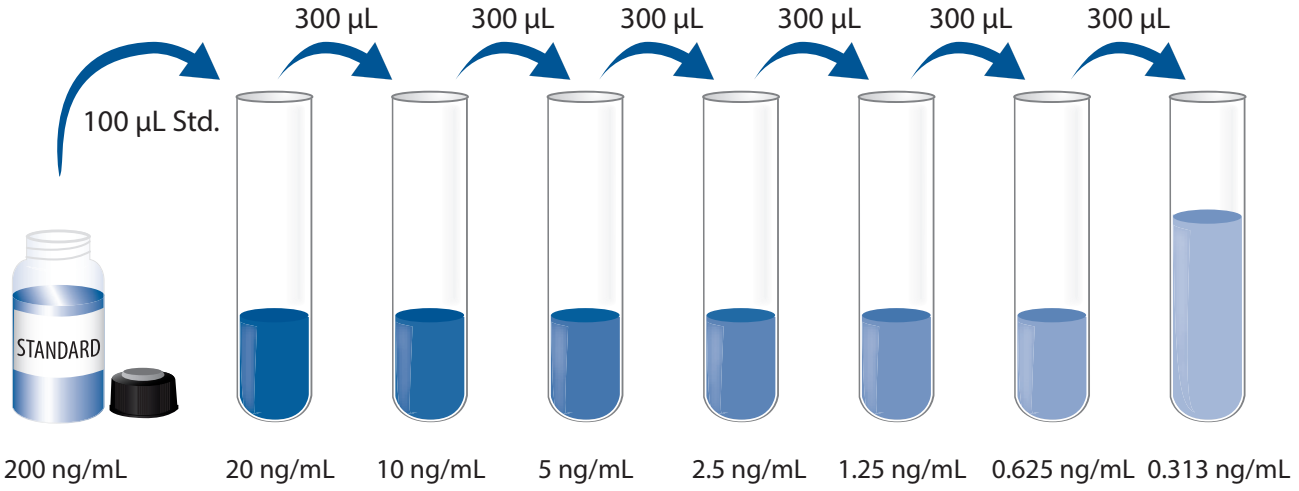
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

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

Human E-Cadherin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human E-Cadherin Standard with deionized or distilled water. This reconstitution produces a stock solution of 200 ng/mL. Mix the standard by inverting the vial 1-2 times to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes prior to making dilutions.

Note: *Do not use rocker or vortex.*

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:5) into the 20 ng/mL tube. Pipette 300 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 20 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 standards, controls, and samples be assayed in duplicate.

Note: *E-Cadherin is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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 RD1-78 to each well. *Assay Diluent RD1-78 may contain crystals. Mix well before use.*
4. Add 50 µL of standard, control, or sample* per well. Ensure sample addition is uninterrupted and completed within 15 minutes. 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 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 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 200 µL of Human E-Cadherin 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 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. 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

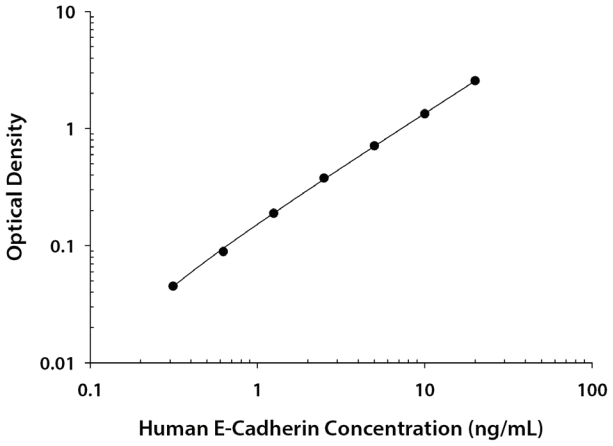
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 human E-Cadherin 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.

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	0.046 0.054	0.050	—
0.313	0.093 0.097	0.095	0.045
0.625	0.133 0.145	0.139	0.089
1.25	0.238 0.239	0.239	0.189
2.5	0.424 0.429	0.427	0.377
5	0.754 0.769	0.762	0.712
10	1.371 1.401	1.386	1.336
20	2.601 2.606	2.604	2.554

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)	2.18	6.49	13.1	2.31	6.67	13.3
Standard deviation	0.18	0.29	0.72	0.24	0.57	0.88
CV (%)	8.3	4.5	5.5	10.4	8.5	6.6

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	106	98-116%

LINEARITY

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

		Cell culture media (n=4)	Serum (n=4)	Heparin plasma (n=4)	Saliva (n=4)	Urine (n=4)
1:2	Average % of Expected	101	104	103	90	110
	Range (%)	99-103	99-112	101-108	85-95	106-112
1:4	Average % of Expected	99	100	98	87	109
	Range (%)	96-105	92-105	94-103	82-90	101-114
1:8	Average % of Expected	101	98	100	89	102
	Range (%)	92-106	84-108	91-109	———	88-113
1:16	Average % of Expected	99	96	95	101	97
	Range (%)	91-108	81-105	91-100	———	85-110

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human E-Cadherin ranged from 0.007-0.090 ng/mL. The mean MDD was 0.039 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 E-Cadherin/Fc Chimera produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human E-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=37)	37.3	21.5-59.3	9.26
Heparin Plasma (n=37)	36.4	21.5-60.3	9.55
Urine (n=15)	4.38	1.66-13.2	3.02
Saliva (n=5)	18.8	4.88-65.0	———

Cell Culture Supernates - The following cell lines were tested for the presence of human E-Cadherin.

Cell Line	Values (ng/mL)
MCF-7 human breast cancer growth media	8.60
MCF-7 human breast cancer w/trypsin	6.08
DLD clone 2C2 human colon adenocarcinoma w/ serum	3.68
DLD clone 2C2 human colon adenocarcinoma w/o serum	6.31
JAR human choriocarcinoma stimulated	1.10
JAR human choriocarcinoma unstimulated	ND
HT-29 human colon adenocarcinoma	0.860
T84 human colon carcinoma	0.978
JE-3 human epithelial choriocarcinoma	1.18

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human E-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 recombinant human E-Cadherin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ALCAM
BCAM
Contactin
Cadherin-8
Cadherin-17
Desmoglein-1
Desmoglein-2
DNAM-1
E-Selectin
Galectin-9
ICAM-1
ICAM-2
ICAM-3
L-Selectin
MCAM
NCAM-L1
P-Selectin
PECAM
TROP-2
VCAM-1
VE-Cadherin

Recombinant mouse:

E-Selectin
ICAM-1
ICAM-2
L-Selectin
P-Cadherin
P-Selectin
VCAM-1

Recombinant mouse E-Cadherin does not interfere but does cross-react approximately 5.5% in this assay.

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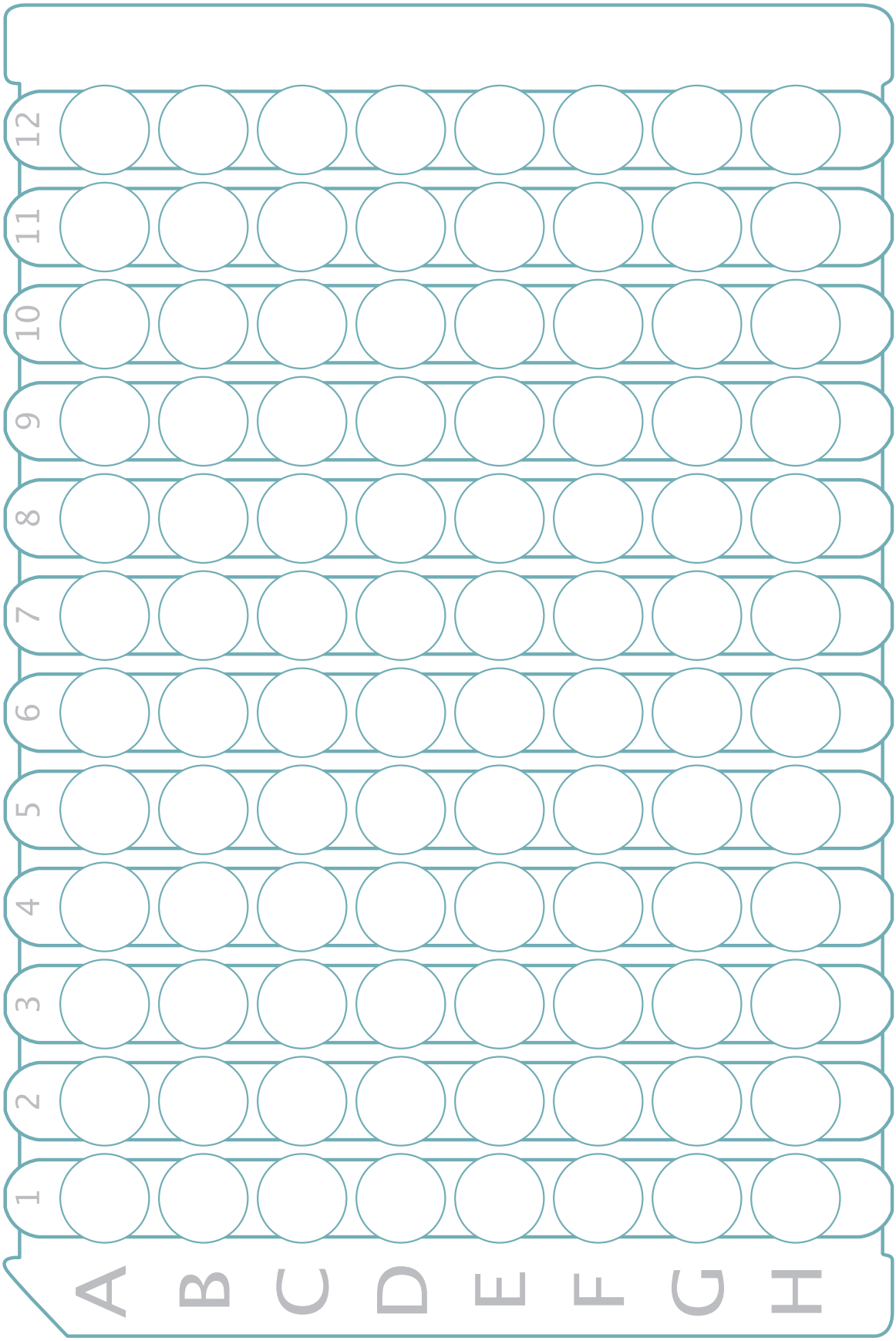
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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