Quantikine™ ELISA

Human E-Cadherin Immunoassay

Catalog Number DCADE0B

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

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

SECTION PAGE

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INTRODUCTION

Epithelial Cadherin (E-Cadherin), also known as ECAD, Cadherin-1 (CDH1), Cell-CAM120/80, and Uvomorulin, is a prototypical member of the cadherin superfamily of cell adhesion molecules. It 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 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 multi-molecular protein complexes that interact with the cytoskeleton and regulate E-Cadherin activity and signal transduction. An 80 kDa Soluble E-Cadherin (sE-Cadherin) isoform is 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 α-, β-, and γ-catenin and members of the Armadillo family. They associate with the catenin binding domain in the cytoplasmic tail of E-Cadherin. Interactions between the E-Cadherin/β- or γ-catenin complex and the F-actin cytoskeleton are mediated directly by α-catenin, 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 affect adhesion depending on the context (14,15).

E-Cadherin also acts 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's complex role in cancer has been correlated with its isoform-dependent effects. Although cadherin switching (downregulation of E-Cadherin/upregulation of N-Cadherin) is considered a hallmark of epithelial to mesenchymal transformation (EMT), controversy remains over the necessity or causality of E-Cadherin (19). Cancer cell dispersion and metastases depend on a loss of cell/cell adhesion. Many *in vitro* studies suggest that tumor invasion is enhanced when E-Cadherin activities are modulated (20). Downregulated or absent E-Cadherin has been associated with many tumor types *in vivo* including pancreatic, gastric, colonic, esophageal, and hepatocellular carcinomas (21-25). Other studies have shown that sE-Cadherin can enhance tumor cell invasion *in vitro*. There is also evidence for elevated sE-Cadherin levels in urine and serum in cancer patients (5, 6, 26, 27). Soluble E-Cadherin has also been associated with increased cancer cell migration, proliferation, and survival (19).

The Quantikine™ Human E-Cadherin Immunoassay is a 3.5 hour solid-phase ELISA designed to measure human E-Cadherin in cell culture supernates, serum, plasma, saliva, and urine. It contains HEK293-expressed recombinant human E-Cadherin 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 monoclonal 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.
- 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	899262	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	899264	2 vials of recombinant human E-Cadherin in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a fresh standard for each assay.
Human E-Cadherin Conjugate	899263	21 mL of a monoclonal 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 RD1-43	895521	11 mL of a buffered protein base containing blue dye and preservatives.	
Calibrator Diluent RD5-26	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	895032	6 mL of 2N 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
- Test tubes for dilution of standards and samples
- Human E-Cadherin Controls (optional; R&D Systems®, Catalog # QC295)

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 or 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: *Citrate plasma has not been validated for use in this assay.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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

Cell culture supernates may require dilution due to high endogenous levels.

Due to high endogenous levels, serum, plasma, and saliva samples require a 20-fold dilution prior to assay. A suggested 20-fold dilution is 20 µL of sample + 380 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

Urine samples require a 2-fold dilution. A suggested 2-fold dilution is 150 µL of sample + 150 µL of Calibrator Diluent RD5-26 (diluted 1:4)*. Further dilution may be required due to high endogenous levels.

Multiple dilutions are recommended for unknown samples.

*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.*

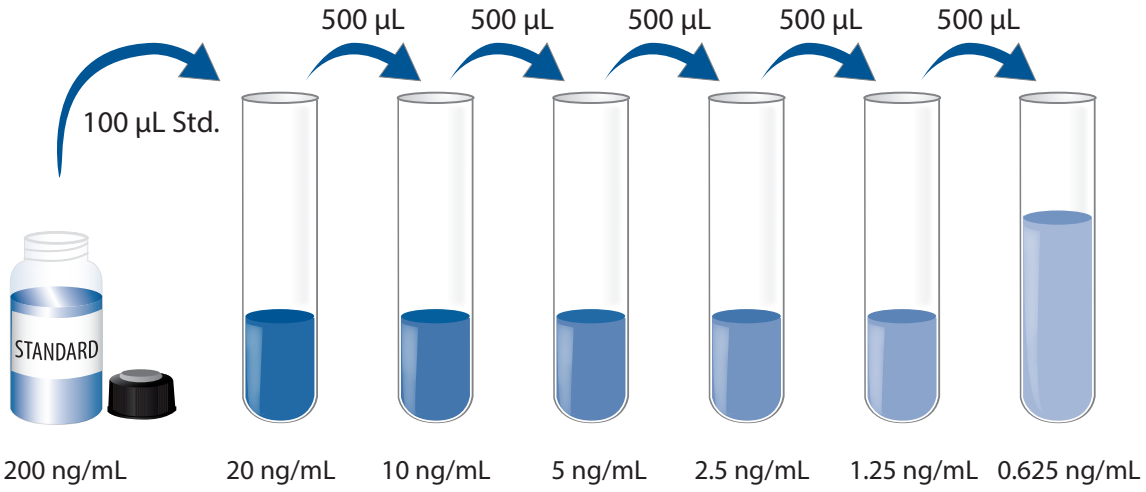
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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 RD5-26 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-26 to 60 mL deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

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 to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-26 (diluted 1:4) into the 20 ng/mL tube. Pipette 500 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 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, 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 RD1-43 to each well.
4. Add 100 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 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 **1 hour** 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 may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

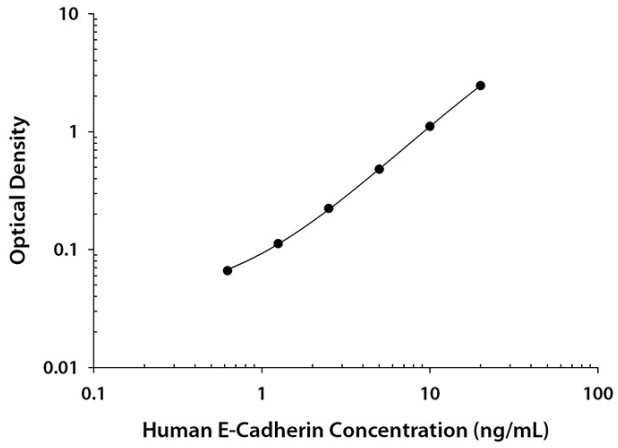
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 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	0.026 0.028	0.027	—
0.625	0.063 0.068	0.066	0.039
1.25	0.110 0.114	0.112	0.085
2.5	0.217 0.229	0.223	0.196
5	0.466 0.493	0.480	0.453
10	1.094 1.121	1.108	1.081
20	2.404 2.509	2.457	2.430

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)	2.42	7.06	14.6	2.19	6.55	14.2
Standard deviation	0.047	0.145	0.266	0.136	0.368	0.845
CV (%)	1.9	2.1	1.8	6.2	5.6	6.0

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	112	103-121
Saliva* (n=4)	100	86-109
Urine* (n=4)	106	100-111

*Samples were diluted prior to assay.

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 supernates* (n=4)	Cell culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Saliva* (n=4)	Urine* (n=4)
1:2	Average % of Expected	99	89	98	97	96	100	93
	Range (%)	93-104	86-93	95-99	92-102	96-97	97-104	87-96
1:4	Average % of Expected	97	86	94	95	92	102	88
	Range (%)	93-100	82-91	91-96	92-96	88-93	97-104	81-92
1:8	Average % of Expected	99	84	94	93	96	106	86
	Range (%)	95-103	81-89	90-97	89-96	91-101	100-111	77-96
1:16	Average % of Expected	103	84	95	96	95	113	88
	Range (%)	100-108	81-89	93-98	90-101	89-103	109-119	79-101

*Samples were diluted prior to assay.

SENSITIVITY

Twenty-seven assays were evaluated and the minimum detectable dose (MDD) of human E-Cadherin ranged from 0.024-0.126 ng/mL. The mean MDD was 0.056 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 HEK293-expressed recombinant human E-Cadherin 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=30)	250	127-492	65.5
EDTA plasma (n=30)	242	131-479	66.5
Heparin Plasma (n=30)	236	116-479	64.9
Urine (n=10)	60.6	3.03-121	33.3
Saliva (n=10)	145	21.4-278	99.8

Cell Culture Supernates:
MCF-7 cells were cultured in a 1:1 mixture of DMEM/Kaighn's F12 supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until nearly confluent. An aliquot of cell culture supernate was removed and assayed for human E-Cadherin, and measured 9.71 ng/mL.

MDA-MB-468 cells were cultured in RPMI supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until nearly confluent. An aliquot of cell culture supernate was removed and assayed for human E-Cadherin, and measured 12.7 ng/mL.

HT-29 cells were cultured in McCoy's 5A supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 ug/mL streptomycin sulfate until nearly confluent. An aliquot of cell culture supernate was removed and assayed for human E-Cadherin, and measured 31.2 ng/mL.

DLD-1 cells were cultured in RPMI supplemented with 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 ug/mL streptomycin sulfate until nearly confluent. An aliquot of cell culture supernate was removed and assayed for human E-Cadherin, and measured 22.2 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human E-Cadherin.

The factors listed below were prepared at 200 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 200 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:

ADAM-10
AJAP-1
β-Catenin
Desmoglein-1
Desmoglein-2
DDR1
Eph A4
Fyn
HDAC-1
HDAC-2
KLRG1
PTPRM
PTPRT

Other recombinants

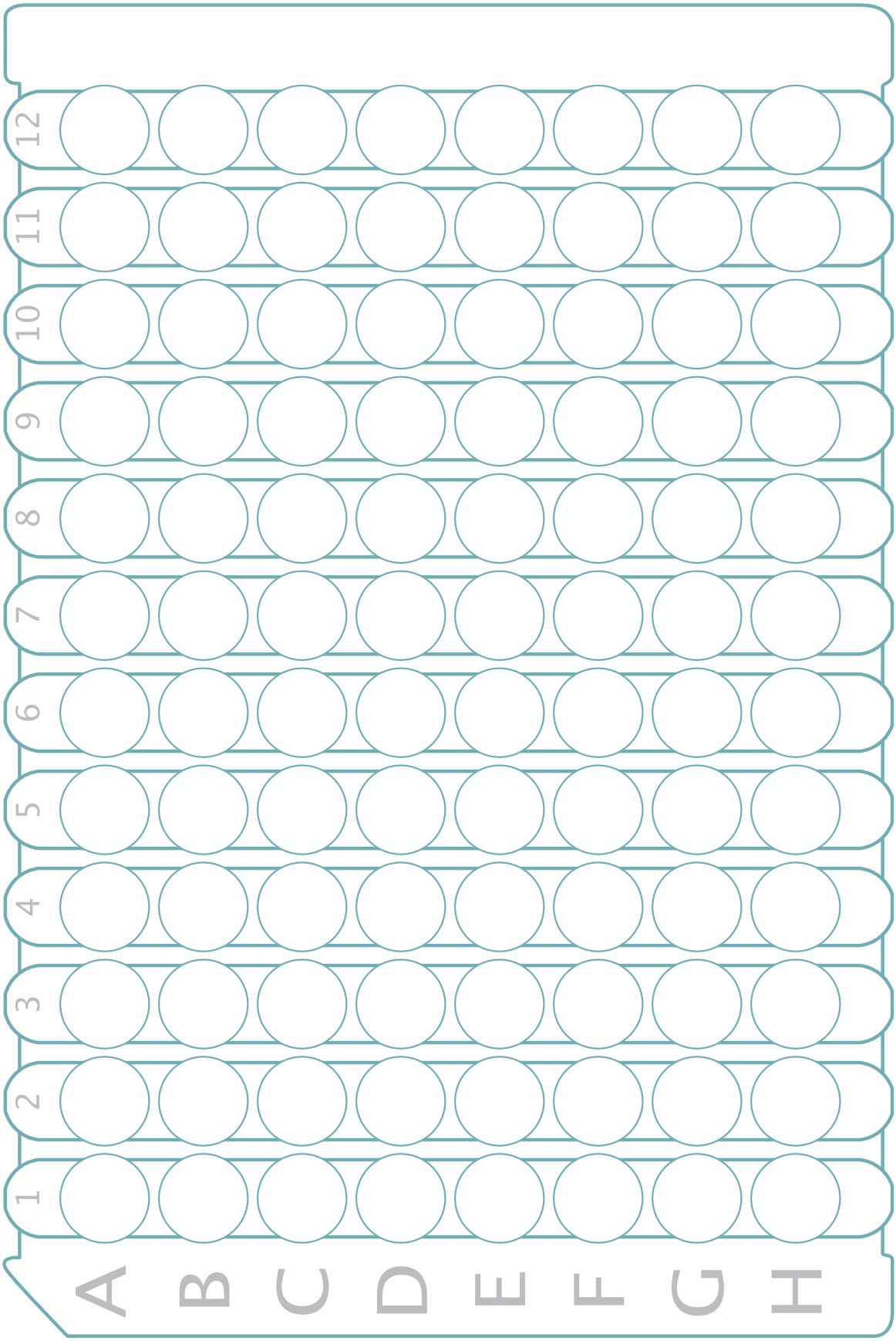
mouse E-Cadherin
rat Cadherin-1

REFERENCES

1. Rimm, D.L. and J.S. Morrow (1994) Biochem. Biophys. Res. Commun. **200**:1754.
2. Lewis, J.E. (1994) J. Cell Sci. **107**:3615.
3. Overduin, M. (1995) Science **267**:386.
4. Pokutta, S. (1994) Eur. J. Biochem. **223**:1019.
5. Noë, V. (2000) J. Cell Sci. **114**:111.
6. Ryniers, F. (2002) Biol. Chem. **383**:159.
7. Vasioukhin, V. (2000) Cell **100**:209.
8. Ikeda, W. (1999) J. Cell Biol. **146**:1117.
9. Pokutta, S. (2002) J. Biol. Chem. **277**:18868.
10. Imamura, Y. (1999) J. Cell Biol. **144**:1311.
11. Kuroda, S. (1998) Science **281**:832.
12. Roura, S. (1999) J. Biol. Chem. **274:**36734.
13. Yap, A.S. (1998) J. Cell Biol. **141**:779.
14. Ohkubo, T. and M. Ozawa (1999) J. Biol. Chem. **274**:21409.
15. Thoreson, M.A. (2000) J. Cell Biol. **148**:189.
16. Kovacs, E.M. (2002) J. Biol. Chem. **277**:6708.
17. Goodwin, M. (2003) J.Biol. Chem. **278**:20533.
18. Kovacs, E.M. (2002) Curr. Biol. **12**:379.
19. Loh, C-Y. (2019) Cells. **8**:1118
20. Mareel, M. (1997) J. Cell Physiol. **173**:271.
21. Weinel, R. (1996) Int. J. Pancreatol. **19**:25.
22. Matsuura, K. (1992) Br. J. Cancer **66**:1122.
23. Dorudi, S. (1993) Am. J. Pathol. **142**:981.
24. Zhao, X-J. (2003) World J. Gastroenterol. **9**:225.
25. Matsumura, T. (2001) Clin. Cancer Res. **7**:594.
26. Katayama, M. (1994) Br. J. Cancer **69**:580.
27. Banks, R.E. (1995) J. Clin. Pathol. **48**:179.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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