Quantikine® ELISA

Human ICAM-1/CD54 Allele-specific Immunoassay

Catalog Number DCD540
SCD540
PDCD540

For the quantitative determination of human soluble Intercellular Adhesion Molecule 1 (ICAM-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.

A polymorphism exists in the human ICAM-1 sequence. The detection antibody used in this kit is allele-specific. It recognizes only the wild type ICAM-1 and does not detect the K29M, Kilifi mutation, ICAM-1. The Kilifi mutation variant is present in people of native-African descent at a frequency reported as high as 20% and predisposes to cerebral malaria (Fernandex-Reyes, D. et al. (1997) Hum. Mol. Genet. **6:1357).*

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

Intercellular Adhesion Molecule 1 (ICAM-1), also known as CD54, is a nearly ubiquitous transmembrane glycoprotein that plays a key role in leukocyte migration and activation (1, 2). Human ICAM-1 contains five Ig-like domains in its extracellular domain (ECD) and associates into non-covalently linked dimers (3, 4). Soluble forms of monomeric and dimeric ICAM-1 (sICAM-1) can be generated via proteolytic cleavage by cathepsin G, elastase, MMP-9, MMP-14/MT1-MMP, and TACE/ADAM17 (5-8). In the mouse, alternate splicing generates isoforms that lack particular Ig-like domains and are differentially sensitive to proteolysis (5). Within the ECD, human ICAM-1 shares 53% amino acid sequence identity with mouse and rat ICAM-1.

The principal binding partners of ICAM-1 are the leukocyte integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) (9-11). The multivalency of dimeric ICAM-1 increases its strength of interaction with LFA-1 (9, 10). ICAM-1 also binds several non-integrin ligands including CD43/sialophorin, fibrinogen, hyaluronan, rhinoviruses, and *Plasmodium falciparum*-infected erythrocytes (12-16). At sites of inflammation, ICAM-1 is upregulated on endothelial and epithelial cells where it mediates the adhesion and paracellular migration of leukocytes expressing activated LFA-1 and Mac-1 (17-20). ICAM-1 ligation prolongs antigen presentation by dendritic cells and promotes T cell proliferation and cytokine release (21-23). ICAM-1 activation also participates in angiogenesis, wound healing, and bone metabolism (24-26).

Soluble ICAM-1 has been reported in serum, cerebrospinal fluid, urine, and bronchoalveolar lavage fluid (2, 27-31). Elevated levels of sICAM-1 in these fluids are associated with cardiovascular disease, type 2 diabetes, organ transplant dysfunction, oxidative stress, abdominal fat mass, hypertension, liver disease, and certain malignancies (32-40). sICAM-1 promotes angiogenesis and serves as an indicator of vascular endothelial cell activation or damage (41, 42). It also functions as an inhibitor of transmembrane ICAM-1 mediated activities such as monocyte adhesion to activated endothelial cells and sensitivity of tumor cells to NK cell-mediated lysis (7, 8).

The Quantikine Human ICAM-1/CD54 Allele-specific immunoassay is a 2.0 hour solid phase ELISA designed to measure ICAM-1 in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human ICAM-1 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human ICAM-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 for naturally occurring human ICAM-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for wild type human ICAM-1 has been pre-coated onto a microplate. Standards, samples, controls, and conjugate are pipetted into the wells and any ICAM-1 present is sandwiched by the immobilized antibody and the enzyme-linked monoclonal antibody specific for human wild type ICAM-1. Following a wash to remove any unbound substances and/or antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of ICAM-1 bound. 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 the 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.
- 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 #	CATALOG # DCD540	CATALOG # SCD540	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human ICAM-1 Microplate	893602	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human wild type and variant ICAM-1.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human ICAM-1 Conjugate	893603	1 vial	6 vials	12 mL/vial of monoclonal antibody specific for human wild type ICAM-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human ICAM-1 Standard	893604	1 vial	6 vials	Recombinant human ICAM-1 in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Calibrator Diluent RD5-7	895045	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

DCD540 contains sufficient materials to run an ELISA on one 96 well plate.
SCD540 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDCD540). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

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.
- 500 mL graduated cylinder.
- 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 ICAM-1 Controls (optional; R&D Systems, Catalog # QC105).

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. 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 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.*

SAMPLE PREPARATION

Serum and plasma samples require a 20-fold dilution. A suggested 20-fold dilution is 20 µL of sample + 380 µL of Calbrator Diluent RD5-7.

REAGENT PREPARATION

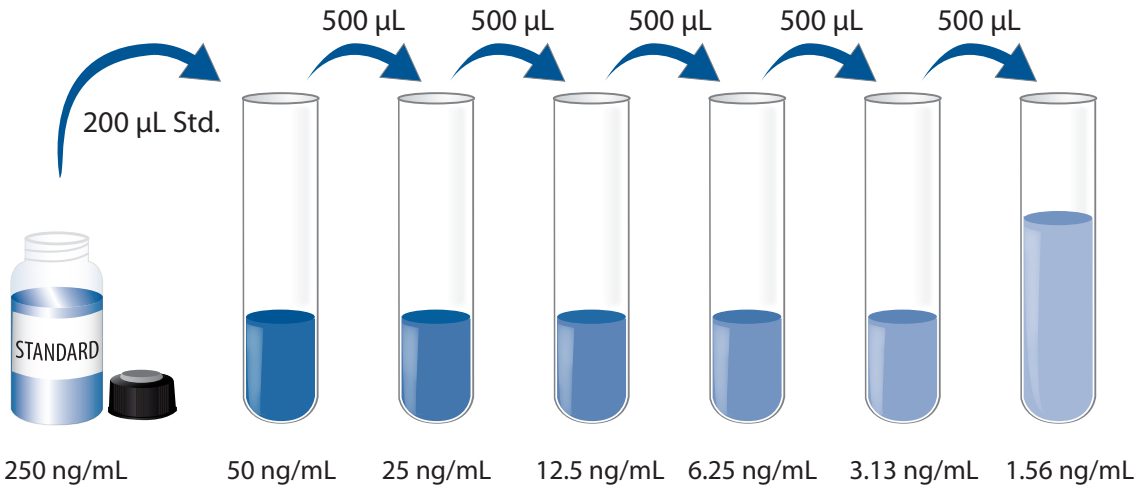
Bring all reagents to room temperature before use.

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.

Human ICAM-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human ICAM-1 Standard with deionized or distilled water. This reconstitution produces a stock solution of 250 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 800 µL of Calibrator Diluent RD5-7 into the 50 ng/mL tube. Pipette 500 µL of Calibrator Diluent RD5-7 into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50 ng/mL standard serves as the high standard. Calibrator Diluent RD5-7 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, samples, and controls 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 Human ICAM-1 Conjugate to each well.
4. Add 100 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 1.5 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 Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the bench top. Protect from light.**
7. 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.
8. 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

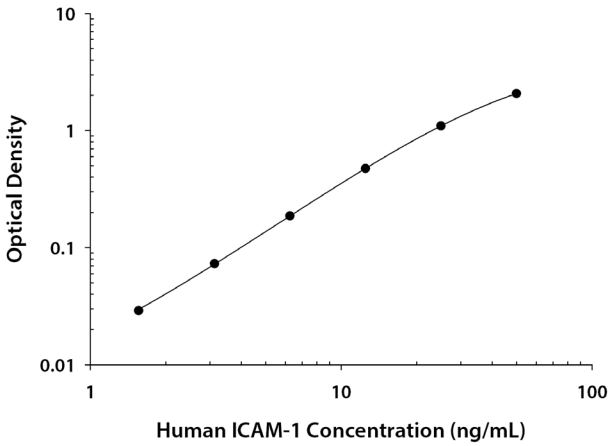
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human ICAM-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

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	0.008 0.011	0.010	—
1.56	0.037 0.041	0.039	0.029
3.13	0.082 0.084	0.083	0.073
6.25	0.196 0.198	0.197	0.187
12.5	0.475 0.492	0.484	0.474
25	1.095 1.116	1.106	1.096
50	2.062 2.100	2.081	2.071

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	20	20	20
Mean (ng/mL)	4.61	12.1	19.6	4.77	12.5	20.5
Standard deviation	0.17	0.63	0.98	0.32	0.67	0.90
CV (%)	3.7	5.2	5.0	6.7	5.4	4.4

RECOVERY

The recovery of human ICAM-1 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	104	90-109%

LINEARITY

To assess the linearity of the assay, samples spiked with or containing high concentrations of human ICAM-1 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	97	101	105	100
	Range (%)	94-101	97-103	104-108	95-104
1:4	Average % of Expected	99	102	104	101
	Range (%)	96-106	99-104	101-106	90-109
1:8	Average % of Expected	95	99	100	104
	Range (%)	92-98	92-108	94-105	99-107

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

SENSITIVITY

Forty-six assays were evaluated and the minimum detectable dose (MDD) of human ICAM-1 ranged from 0.049-0.254 ng/mL. The mean MDD was 0.096 ng/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.

CALIBRATION

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

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human ICAM-1 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=54)	208	98.8-320	40.7
EDTA plasma (n=54)	211	100-307	42.9
Heparin plasma (n=54)	204	106-337	41.6

Cell Culture Supernates - Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal calf 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. Aliquots of the cell culture supernate were removed and assayed for human ICAM-1. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant human ICAM-1.

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 ICAM-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ALCAM
BCAM
Cadherin-8
Cadherin-11
CD31/PECAM-1
CHL-1
Contactin-1
Contactin-2
Contactin-4
DC-SIGNR
Desmoglein-1
Desmoglein-2
DNAM-1
E-Cadherin
Endocan
EpCAM
E-Selectin
Galectin-1
Galectin-2
Galectin-3
Galectin-4
Galectin-7
Galectin-8
ICAM-2
ICAM-3
ICAM-5
JAM-A
JAM-B
JAM-C
LOX-1
L-Selectin
MCAM
N-Cadherin
NCAM-1
NrCAM
P-Cadherin
P-Selectin
Siglec-2
Siglec-3
Siglec-5
Siglec-6
Siglec-7
Siglec-9
Siglec-10
TROP-2
VCAM-1
VE-Cadherin

Recombinant mouse:

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

Recombinant rat:

ICAM-1
E-Selectin
L-Selectin
MAG

This assay does not recognize a natural variant of intercellular adhesion molecule-1 (ICAM-1^{Kilifi}). This polymorphism at K29/M in the N-terminal domain of ICAM-1 is present in African-Americans at a frequency reported as high as 20% and predisposes to cerebral malaria (43-45). An internal study of 30 African-American samples found only one sample (3.3%) non-detectable.

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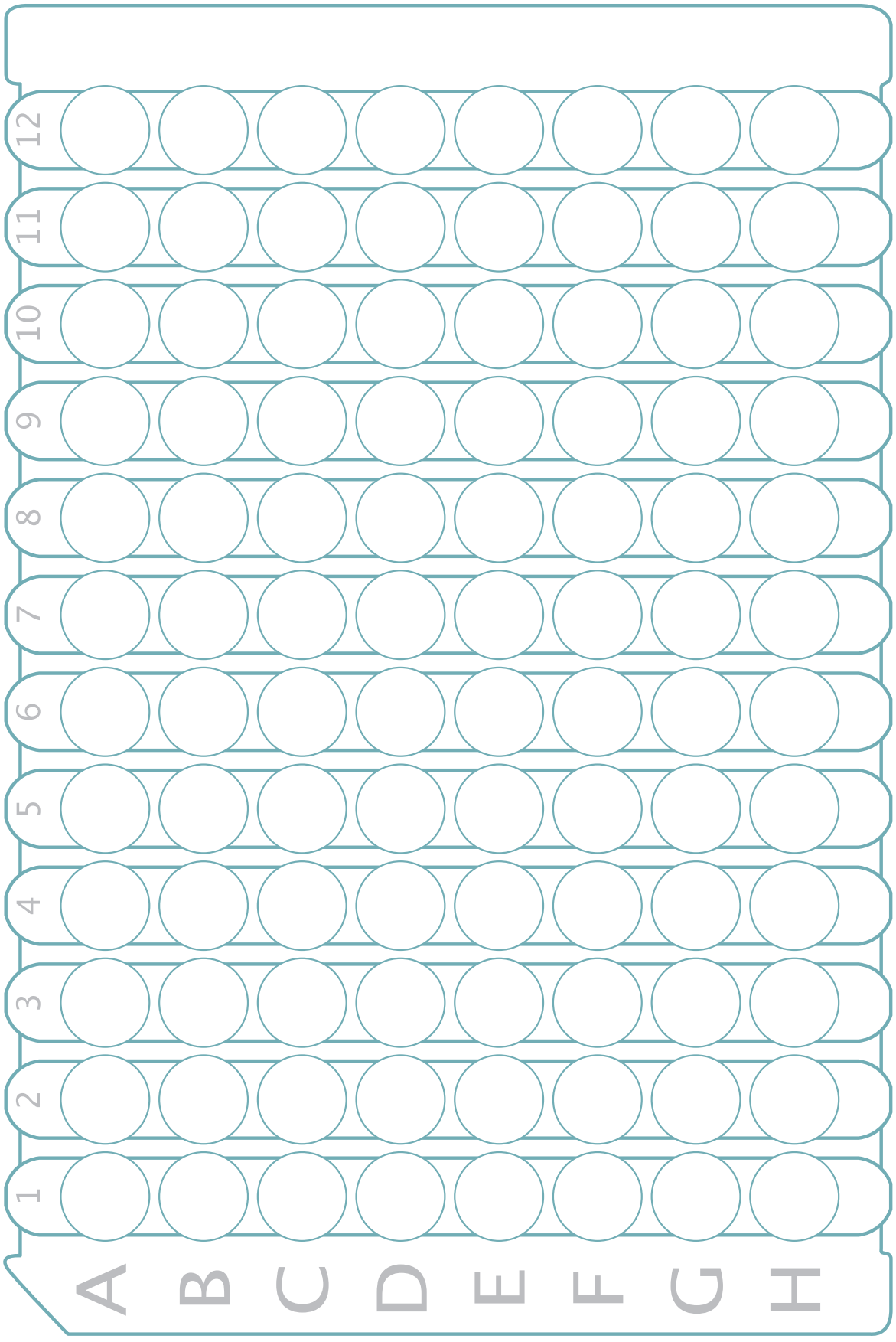
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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