bio-techne®

R&D SYSTEMS

Quantikine™ ELISA

Human E-Selectin/CD62E Immunoassay

Catalog Number DSLE00
SSLE00
PDSLE00

For the quantitative determination of human 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.

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Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956
FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

E-Selectin (also known as Endothelial Leukocyte Adhesion Molecule-1, ELAM-1, or CD62E) is a 115 kDa, type I transmembrane glycoprotein expressed only on endothelial cells and only after activation by inflammatory cytokines (IL-1β or TNF-α) or endotoxins (1-4). Expression is transitory, reaching a maximum within about 6 hours of stimulation and then declining with shedding of soluble E-Selectin (1-5). Cell-surface E-Selectin is a mediator of the rolling attachment of leukocytes to the endothelium, an essential step in extravasation of leukocytes at the site of inflammation (1-6) thereby playing a key role in localized inflammatory response. E-Selectin is believed to be particularly important in inflammation involving the skin (4).

The extracellular part of E-Selectin includes a calcium-dependent C2-type lectin domain, an epidermal growth factor (EGF) domain, and six repeats of a complement-regulatory-protein-like sequence (1-4). E-Selectin binds sialyl Lewis X (sLe^x), a sialic acid-galactose-N-acetylglucosamine-fructose tetrasaccharide, but the actual recognition is thought to be for a specific presentation of those glycosyl units in a precise three-dimensional configuration on a specific glycoprotein rather than for that particular carbohydrate (4).

E-Selectin is found in the blood of healthy individuals (7, 8), probably arising from proteolytic cleavage of the surface-expressed molecule (5, 9). Elevated levels of E-Selectin in serum have been reported in a variety of pathological conditions (8, 10-14). Although it might be anticipated that E-Selectin would suppress leukocyte migration by competing with surface-associated E-Selectin, it may actually activate neutrophils and act as a pro-inflammatory agent (15).

The Quantikine™ Human E-Selectin/CD62E Immunoassay is a 4.5 hour solid phase ELISA designed to measure human E-Selectin in cell culture supernates, serum, and plasma. It contains CHO cell-expressed recombinant human E-Selectin and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human 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 for natural human E-Selectin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human E-Selectin has been pre-coated onto a microplate. Standards 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 human 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 and color develops in proportion to the amount of E-Selectin 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 #	CATALOG # DSLE00	CATALOG # SSLE00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human E-Selectin Microplate	893122	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human 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.*
Human E-Selectin Conjugate	893123	1 vial	6 vials	21 mL/vial of a monoclonal antibody specific for human E-Selectin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human E-Selectin Standard	893124	1 vial	6 vials	Recombinant human E-Selectin in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1W	895117	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD6-11	895489	2 vials	8 vials	21 mL/vial of a buffered protein solution with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives. *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 2N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DSLE00 contains sufficient materials to run an ELISA on one 96 well plate.
SSLE00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDSLE00). Refer to the PharmPak Contents section 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
- Test tubes for dilution of standards and samples
- Human E-Selectin Controls (optional; R&D Systems®, Catalog # QC236)

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human E-Selectin Microplate	893122	50 plates
Human E-Selectin Conjugate	893123	50 vials
Human E-Selectin Standard*	893124	25 vials
Calibrator Diluent RD6-11**	895489	50 vials
Assay Diluent RD1W	895117	50 vials
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Wash Buffer Concentrate	895126	9 bottles
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets
Package Insert	751599	2 booklets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

*** If purchasing controls (R&D Systems, Catalog # QC236) additional vials of Calibrator Diluent RD6-11 may be required for reconstitution.*

PRECAUTIONS

Calibrator Diluent RD6-11 contains 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 SDS 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 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.

Note: *EDTA plasma is not recommended for use in this assay, because E-Selectin is a* Ca^{2+}*-dependent molecule.*

SAMPLE PREPARATION

Cell culture supernate samples require a 2-fold dilution. A suggested 2-fold dilution is 150 µL of sample + 150 µL of Calibrator Diluent RD6-11.

Serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 30 µL of sample + 270 µL of Calibrator Diluent RD6-11.

REAGENT PREPARATION

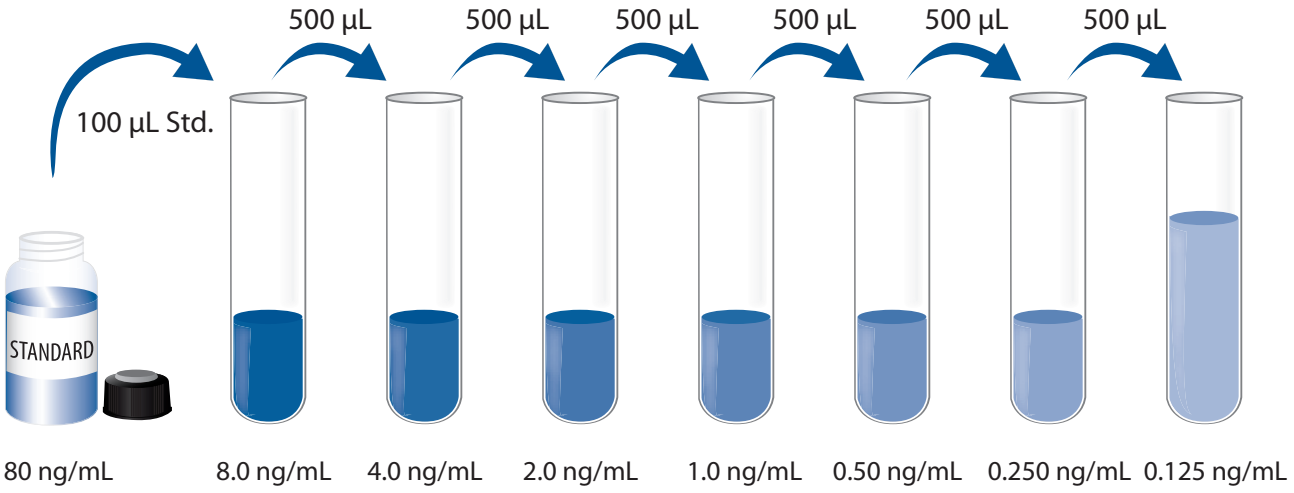
Bring all reagents to room temperature before use.

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 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.

Human E-Selectin Standard - Refer to the vial label for reconstitution volume. Reconstitute the Human E-Selectin Standard with deionized or distilled water. This reconstitution produces a stock solution of 80 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 RD6-11 into the 8.0 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 8.0 ng/mL standard serves as the high standard. Calibrator Diluent RD6-11 serves as the zero standard (0 ng/mL).



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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.

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 RD1W 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. 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-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 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

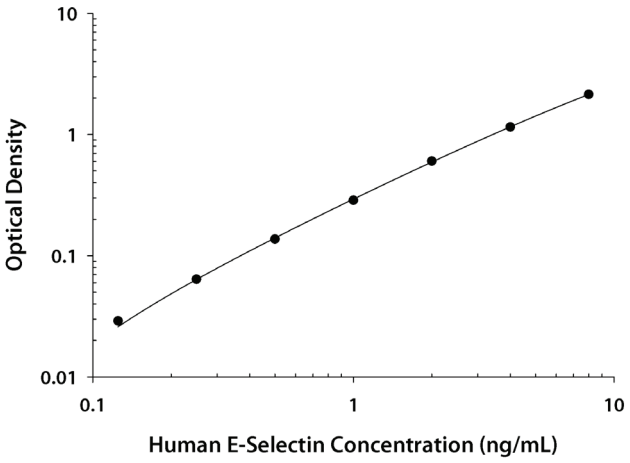
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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

Because 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 0.008	0.008	—
0.125	0.036 0.038	0.037	0.029
0.250	0.069 0.074	0.072	0.064
0.50	0.139 0.150	0.145	0.137
1.0	0.290 0.300	0.295	0.287
2.0	0.569 0.653	0.611	0.603
4.0	1.138 1.183	1.161	1.153
8.0	2.122 2.175	2.149	2.141

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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)	1.6	2.9	4.7	1.5	2.9	4.6
Standard deviation	0.11	0.17	0.24	0.11	0.25	0.35
CV (%)	6.9	5.9	5.1	7.3	8.6	7.6

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	103	98-110%
Serum (n=4)	96	85-106%
Heparin plasma (n=4)	94	85-107%
Citrate plasma (n=4)	96	85-107%

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human E-Selectin ranged from 0.003-0.027 ng/mL. The mean MDD was 0.009 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.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human E-Selectin were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay as directed in the Sample Preparation section.

		Cell culture media (n=4)	Serum (n=4)	Heparin plasma (n=4)	Citrate plasma (n=4)
1:2	Average % of Expected	96	99	105	97
	Range (%)	94-99	95-103	100-112	89-103
1:4	Average % of Expected	99	101	108	101
	Range (%)	94-104	96-105	105-112	92-111
1:8	Average % of Expected	101	104	107	98
	Range (%)	94-108	100-112	93-114	91-102
1:16	Average % of Expected	90	107	102	93
	Range (%)	85-96	104-110	95-108	87-98

CALIBRATION

This immunoassay is calibrated against a highly purified CHO cell-expressed recombinant human E-Selectin produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human E-Selectin 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=35)	37.0	17.9-79.2	14.9
Heparin plasma (n=34)	32.0	15.6-66.4	12.6
Citrate plasma (n=35)	29.6	13.0-51.3	9.4

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

SPECIFICITY

This assay recognizes natural and recombinant human 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 160 ng/mL in a mid-range recombinant human E-Selectin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ALCAM
BCAM
Cadherin-8
Contactin-1
DC-SIGNR
Desmoglein-1
Desmoglein-2
E-Cadherin
Galectin-1
Galectin-2
Galectin-4
Galectin-7
Galectin-8
ICAM-1
ICAM-2
ICAM-3
JAM-A
JAM-B
JAM-C
LAMP
L-Selectin
N-Cadherin
NCAM-L1
P-Cadherin
PECAM-1
P-Selectin
Siglec-3
Siglec-5
Siglec-7
Siglec-9
VCAM-1
VE-Cadherin

Recombinant mouse:

ALCAM
E-Cadherin
E-Selectin
Galectin-1
Galectin-3
Galectin-7
ICAM-1
ICAM-2
ICAM-5
JAM-A
JAM-B
JAM-C
L-Selectin
MadCAM-1
P-Cadherin
P-Selectin
VCAM-1

Recombinant rat:

E-Selectin
ICAM-1
Siglec-4

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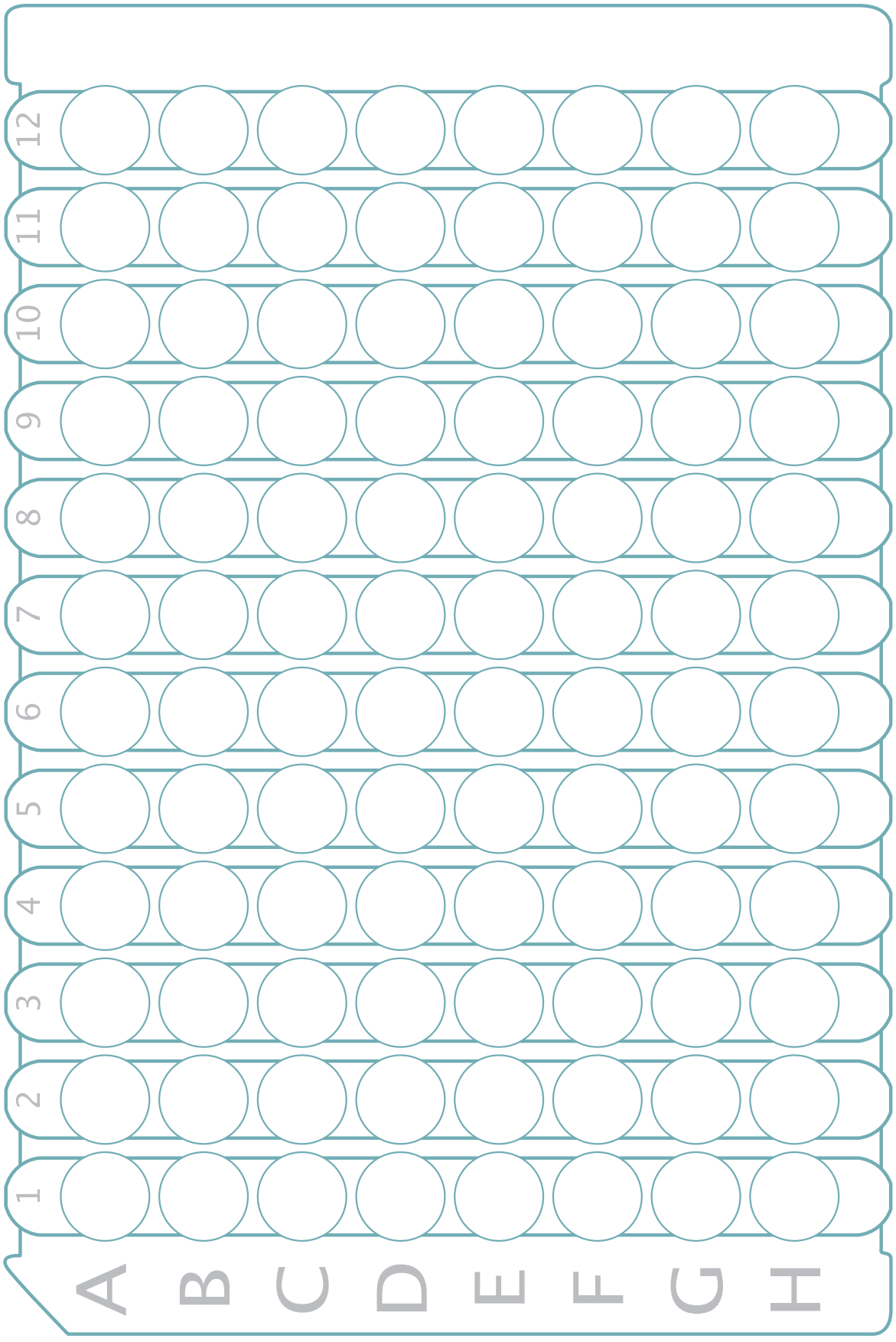
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES

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