Quantikine® ELISA

Human SLPI Immunoassay

Catalog Number DPI00

For the quantitative determination of human Secretory Leukocyte Protease Inhibitor (SLPI) concentrations in cell culture supernates, serum, plasma, and urine.

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

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

Secretory Leukocyte Protease Inhibitor (SLPI), also known as ALP, BLPI, HUSI-1, CUSI, MPI, and Protease Inhibitor WAP4 is a 12-14 kDa member of the Whey Acidic Protein (WAP) family, ALP superfamily of proteins (1-4). It is a non-glycosylated, highly basic, cysteine-rich, monomeric secreted protein that plays a key role in homeostasis (5, 6). Human SLPI is synthesized as a 132 amino acid (aa) precursor that contains a 25 aa signal sequence and a 107 aa mature segment. The mature region contains two WAP domains (aa 28-76 and 82-130), each of which possess four intrachain disulfide bonds (1, 2, 7, 8). The N-terminal domain is associated with anti-inflammatory activity, while the C-terminal domain via aa 67-74 mediates anti-protease activity (9, 10). SLPI will undergo extracellular proteolytic cleavage. Enzymes that act on SLPI include cathepsin-L, -B and -S. Cleavage occurs either between Ser15-Glu17, or Thr67-Tyr68, the latter of which is subsequently followed by further cleavage of aa 68-74 (9, 10). In either case, there is a loss of functional activity associated with each domain. Mature human SLPI shares 53% and 65% aa sequence identity with mouse and porcine SLPI, respectively. SLPI is secreted by multiple cell types, including placental trophoblasts (11), neutrophils and keratinocytes (12), Clara cells and pseudostratified respiratory epithelium (8), adipocytes (13), type II Greater alveolar cells and macrophages (14), plus serous cells of the salivary glands and mucous gland cells of the gut submucosa (5).

SLPI has multiple functions associated with it. It is perhaps best known for its anti-protease activity that involves the neutralization of NE/neutrophil elastase, trypsin, chymotrypsin, cathepsin G, tryptase and chymase (3). This activity serves to retain ground substance and ECM organization during times of inflammation and stress. Notably, SLPI is considered the key inhibitor of NE. In this regard, it will bind NE and target this complex to a nearby cell wall via its strong positive charge (15). Neutralization may be aided here by an additional interaction of SP-A with SLPI on the cell wall. SP-A protects SLPI from attack by MMPs, prolonging its life (14, 15). SLPI is also known to undergo covalent attachment to ECM through transglutaminase bonding to select WAP-1 domain Lys and Gln residues. This does not impact SLPI neutralizing activity (16). Neutralization does not appear to be limited to the extracellular space, as SLPI in neutrophil granules likely serves as a buffer against endogenous enzyme activity that may be directed against secondary granule contents (12). There are at least four "receptors" for SLPI; annexin II, scramblase-1 and -4, and mannose-based PAMPs. Annexin and scramblase binding may impede HIV infection, while PAMP binding may serve to opsonize microbes (17-19). SLPI also demonstrates antimicrobial activity against Gram positive and negative bacteria, likely acting through its strong positive charge (6).

The Quantikine Human SLPI Immunoassay is a 4.5 hour solid phase ELISA designed to measure SLPI in cell culture supernates, serum, plasma, and urine. It contains *E. coli*-expressed recombinant Human SLPI and antibodies raised against the recombinant factor. The kit has been shown to accurately quantitate recombinant human SLPI. Results obtained using natural human SLPI showed linear curves that were parallel to the curves obtained using the recombinant kit standards. These results indicate that this kit can be used to determine relative mass values for natural human SLPI.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human SLPI has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any SLPI present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human SLPI 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 SLPI 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 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 #	DESCRIPTION	STORAGE OF OPENED/RECONSTITUTED MATERIAL
Human SLPI Microplate	890147	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human SLPI.	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 SLPI Standard	890149	Recombinant human SLPI in a buffered protein base 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 SLPI Conjugate	890148	21 mL of a polyclonal antibody specific for human SLPI conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1Q	895079	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5T	895175	2 vials (21 mL/vial) of a buffered protein base with preservatives.	
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.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards and samples.
- Human SLPI Controls (optional; R&D Systems, Catalog # QC21).

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, 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.

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

SAMPLE PREPARATION

Serum and plasma samples require at least a 20-fold dilution prior to assay. A suggested 20-fold dilution is 15 µL of sample + 285 µL of Calibrator Diluent RD5T.

Urine samples require a 5-fold dilution prior to assay. A suggested 5-fold dilution is 50 µL of sample + 200 µL of Calibrator Diluent RD5T.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

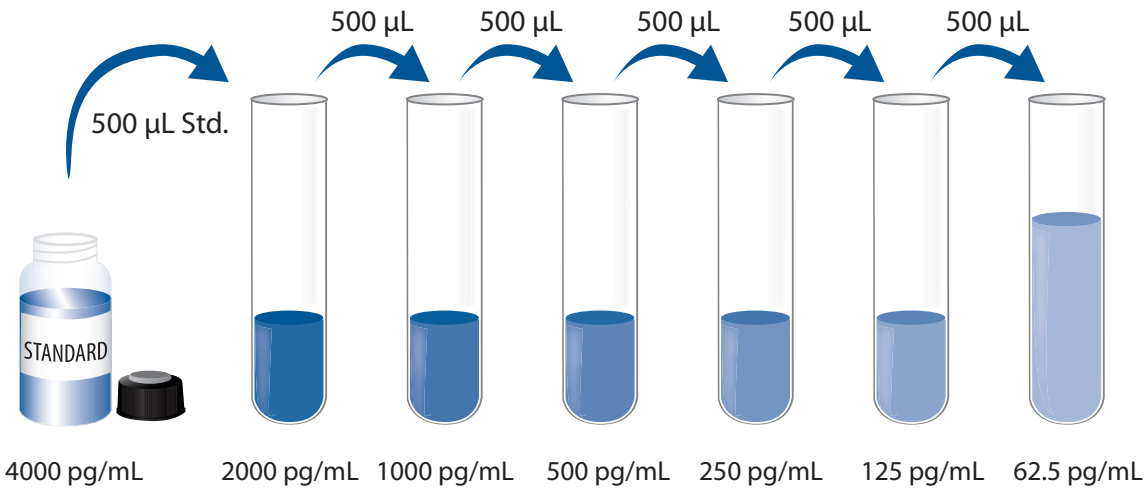
Note: *High levels of SLPI are found in saliva. Take necessary precautions (e.g. mask and gloves) to protect kit reagents.*

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 SLPI Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human SLPI Standard with Calibrator Diluent RD5T. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of Calibrator Diluent RD5T into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human SLPI Standard (4000 pg/mL) serves as the high standard. Calibrator Diluent RD5T serves as the zero standard (0 pg/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.

Note: *High levels of SLPI are found in saliva. Take necessary precautions (e.g. mask and gloves) to protect kit reagents.*

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 RD1Q 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 twice for a total of three 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 toweling.
6. Add 200 µL of Human SLPI 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 20 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 microtiter plate 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

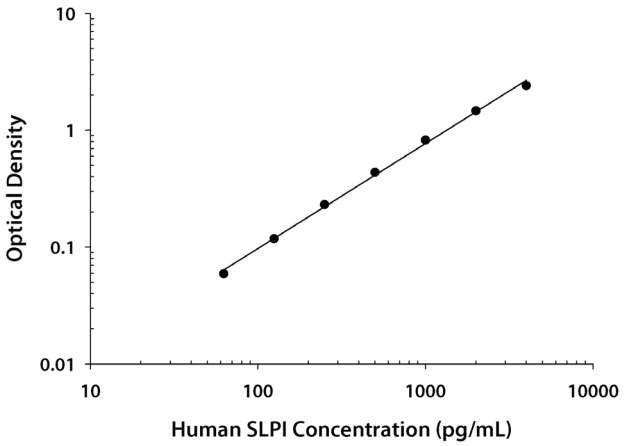
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 a log/log graph. The data may be linearized by plotting the log of the human SLPI concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the 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.



(pg/mL)	O.D.	Average	Corrected
0	0.045 0.043	0.044	—
62.5	0.104 0.102	0.103	0.059
125	0.163 0.160	0.162	0.118
250	0.277 0.273	0.275	0.231
500	0.480 0.481	0.480	0.436
1000	0.858 0.875	0.866	0.822
2000	1.515 1.503	1.509	1.465
4000	2.486 2.419	2.452	2.408

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 (pg/mL)	109	693	2026	188	1174	2157
Standard deviation	8.7	37.1	84.1	15.1	57.5	123
CV (%)	8.0	5.4	4.2	8.0	4.9	5.7

RECOVERY

The recovery of human SLPI was determined in various matrices by mixing high dose samples with low dose samples in ratios of 1:2, 1:1, and 2:1.

Sample Type	Average % Recovery	Range
Cell culture media	94	84-105%
Serum	98	89-104%
EDTA plasma	96	87-103%
Heparin plasma	106	98-116%
Citrate plasma	97	92-102%
Urine	98	92-103%

SENSITIVITY

The minimum detectable dose (MDD) of human SLPI is typically less than 25 pg/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 *E. coli*-expressed recombinant human SLPI produced at R&D Systems.

LINEARITY

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

		Cell culture media (n=1)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Citrate plasma* (n=4)	Urine* (n=4)
1:2	Average % of Expected	111	110	108	112	117	109
	Range (%)	———	107-115	105-112	108-116	113-120	106-110
1:4	Average % of Expected	111	110	106	115	118	109
	Range (%)	———	105-114	101-110	113-117	117-121	105-113
1:8	Average % of Expected	101	105	101	112	115	106
	Range (%)	———	96-112	96-105	107-115	112-119	100-111
1:16	Average % of Expected	98	97	98	105	110	101
	Range (%)	———	91-103	88-121	98-117	106-114	97-105

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

SAMPLE VALUES

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

Sample Type	Mean (pg/mL)	Range (pg/mL)
Serum (n=40)	36,052	27,380-46,880
EDTA plasma (n=40)	39,647	29,180-51,160
Heparin plasma (n=40)	35,882	22,360-47,800
Citrate plasma (n=40)	38,001	26,940-52,920
Urine (n=40)	4001	336-17,425

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1 and 5 days. All samples measured less than the lowest human SLPI standard, 62.5 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human SLPI.

Each of 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 SLPI control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ANG
CNTF
β-ECGF
EGF
Epo
FGF acidic
FGF basic
FGF-4
G-CSF
GM-CSF
GROα
IFN-γ
IGF-I
IGF-II
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-6 R
IL-7
IL-8
IL-9
IL-10
IL-11
LIF
M-CSF
MCP-1
MIP-1α
MIP-1β
OSM
PDGF-AA
PDGF-AB
PDGF-BB
RANTES
SCF
TGF-α
TGF-β1
TGF-β2
TGF-β3
TNF-α
TNF-β
TNF RI
TNF RII

Recombinant mouse:

EGF
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
MIP-1α
MIP-1β
SCF
TNF-α

Other recombinants:

amphibian TGF-β5
chicken TGF-β5

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
human TGF-β1
porcine TGF-β1
porcine TGF-β2
porcine TGF-β1.2

Leukocyte elastase, an enzyme known to be inhibited by SLPI, was tested for cross-reactivity and interference at 250 ng/mL and 1 µg/mL. No cross-reactivity or interference was observed. No cross-reactivity or interference from bovine pancreatic trypsin (1 µg/mL) or chymotrypsin (500 ng/mL) was observed.

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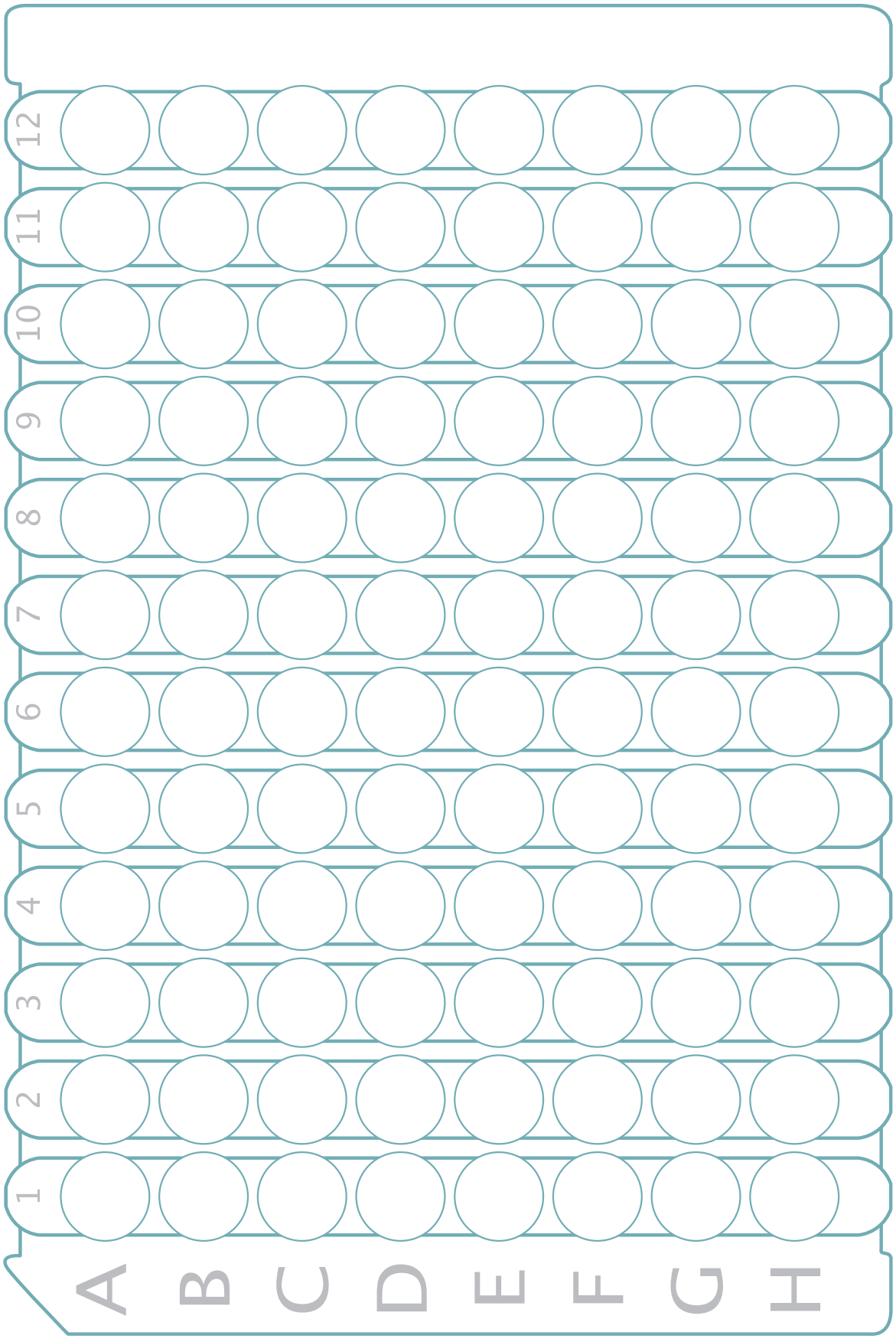
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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