Quantikine™ ELISA

Human Total Serpin E1/PAI-1 Immunoassay

Catalog Number DTSE100

For the quantitative determination of human free and complexed Serpin E1/PAI-1 (Total Serpin E1/PAI-1) 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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INTRODUCTION

Serpin E1, also known as Plasminogen Activator Inhibitor-1 (PAI-1), is an approximately 55 kDa member of the Serpin superfamily of serine protease inhibitors. Serpin E1 is the primary inhibitor of urokinase-type and tissue-type plasminogen activators (uPA and tPA) which convert Plasminogen to Plasmin. The PA-Plasmin system is involved in multiple physiological and pathological processes such as fibrinolysis, tissue fibrosis, angiogenesis, wound healing, tumor cell invasion and metastasis, and obesity (1-3). Serpin E1 is secreted by many cell types including monocytes, platelets, vascular endothelial cells and smooth muscle cells, mature adipocytes, hepatocytes, trophoblast cells, and breast cancer cells (4-12). Its expression is upregulated during inflammation, physical injury, and exposure to Angiotensin II (5, 10, 13-15). Its serum levels are elevated in response to arterial injury, hypoxic exposure, pro-thrombotic disorders, and pregnancy (5, 16-18). Human Serpin E1 shares approximately 80% amino acid sequence identity with mouse and rat Serpin E1, respectively (19, 20).

Secreted Serpin E1 is unstable in its active form and spontaneously converts into a latent, inactive form. Both of these forms circulate in the plasma (5, 7, 21). The active form of Serpin E1 binds and inactivates uPA and tPA (22). It also binds and protects Fibrin from tPA-induced fibrinolysis (23). Serpin E1 thereby inhibits the dissolution of blood clots and plays a protective role against pathological fibrosis (16, 24). In addition, free Serpin E1 binds to the extracellular matrix protein Vitronectin, although complexes of Serpin E1 with plasminogen activators do not (25). Serpin E1 binding prevents the interaction of Vitronectin with Integrins αVβ3 and αVβ5, resulting in inhibition of vascular smooth muscle cell and endothelial cell adhesion, proliferation, and motility (26-29). Serpin E1 can also promote clot formation, arterial re-endothelialization, and neointima formation while protecting against vascular intima thickening (17, 18, 30-32). Through its interactions with matrix proteins and components of the plasminogen system, Serpin E1 can exert either positive or negative effects on angiogenesis (29, 33-35). Serpin E1 binds and induces the internalization of cell surface complexes containing uPA, uPAR, and Integrin αVβ3 (27, 36). Internalization of these complexes is dependent on their association with LRP (37-40).

The Quantikine™ Human Total Serpin E1/PAI-1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Serpin E1 in cell culture supernates, serum, and plasma. It contains *Sf*21-expressed recombinant human Serpin E1 and has been shown to accurately quantitate the recombinant factor free and in complex with uPA, tPA, or vitronectin. Results obtained using natural human Serpin E1 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 Serpin E1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for human Total Serpin E1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Serpin E1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Total Serpin E1 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 Serpin E1 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Total Serpin E1/PAI-1 Microplate	894720	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for human Total Serpin E1.	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 Total Serpin E1/PAI-1 Standard	894722	2 vials of recombinant human Serpin E1 in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human Total Serpin E1/PAI-1 Conjugate	894721	21 mL of polyclonal antibody specific for human Total Serpin E1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-57	895207	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	2 vials (21 mL/vial) of a concentrated buffered protein base with preservatives. *Use undiluted 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
- 500 mL graduated cylinder
- Test tubes for dilution of standards and samples
- Human Total Serpin E1/PAI-1 Controls (optional; R&D Systems®, Catalog # QC209)

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. 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 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.

SAMPLE PREPARATION

Serum samples require a 25-fold dilution. A suggested 25-fold dilution is 20 µL of sample + 480 µL of Calibrator Diluent RD5-26 Concentrate.

Plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 50 µL of sample + 450 µL of Calibrator Diluent RD5-26 Concentrate.

REAGENT PREPARATION

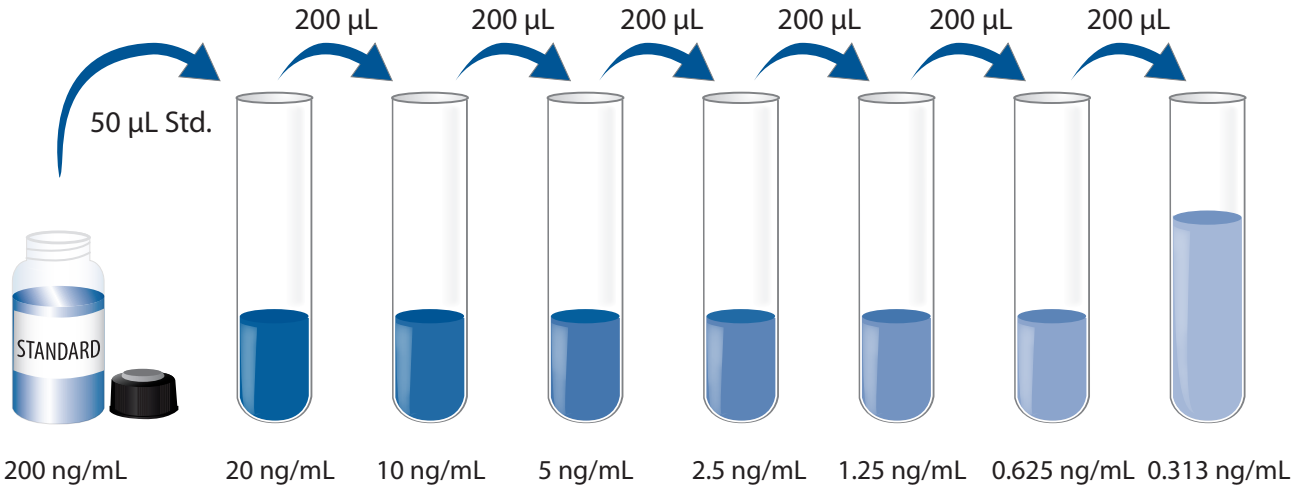
Bring all reagents to room temperature before use.

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 Total Serpin E1/PAI-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Total Serpin E1/PAI-1 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 450 µL of Calibrator Diluent RD5-26 Concentrate into the 20 ng/mL tube. Pipette 200 µL of Calibrator Diluent RD5-26 Concentrate 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 Concentrate 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.

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 50 µL of Assay Diluent RD1-57 to each well.
4. Add 50 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 Total Serpin E1/PAI-1 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 may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

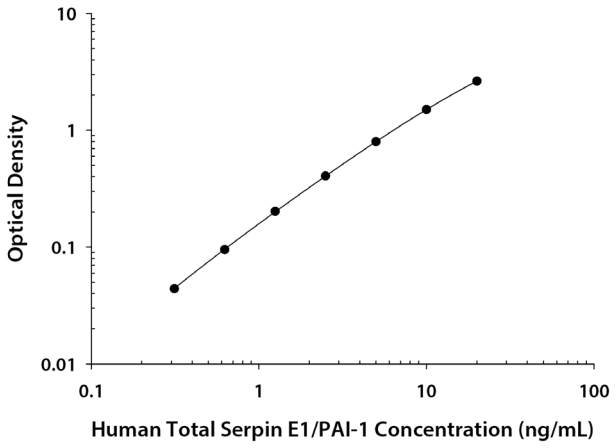
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 Total Serpin E1 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	0.015 0.017	0.016	—
0.313	0.056 0.063	0.060	0.044
0.625	0.107 0.114	0.111	0.095
1.25	0.213 0.222	0.218	0.202
2.5	0.412 0.432	0.422	0.406
5	0.813 0.813	0.813	0.797
10	1.488 1.550	1.519	1.503
20	2.628 2.660	2.644	2.628

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)	1.61	5.05	11.1	1.82	5.28	10.0
Standard deviation	0.097	0.330	0.495	0.196	0.451	0.604
CV (%)	6.0	6.5	4.5	10.8	8.5	6.0

RECOVERY

The recovery of human Total Serpin E1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	105	90-119%
Serum* (n=4)	104	93-116%
EDTA plasma* (n=4)	99	85-113%
Heparin plasma* (n=4)	103	81-116%
Citrate plasma* (n=4)	104	87-118%

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

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human Serpin E1 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. All samples were diluted prior to assay.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Citrate plasma (n=4)
1:2	Average % of Expected	102	106	108	105	103
	Range (%)	99-104	100-113	104-110	96-109	99-106
1:4	Average % of Expected	97	107	102	101	101
	Range (%)	89-106	98-115	101-103	96-105	93-105
1:8	Average % of Expected	95	106	100	100	99
	Range (%)	89-101	100-115	98-101	94-107	93-102
1:16	Average % of Expected	95	103	95	99	95
	Range (%)	92-97	98-112	87-100	93-108	89-103

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of human Total Serpin E1 ranged from 0.005-0.046 ng/mL. The mean MDD was 0.014 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 *Sf*21-expressed recombinant human Serpin E1 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human Serpin E1 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)	82.2	35.8-137	22.8
EDTA plasma (n=35)	20.7	5.00-94.6	15.6
Heparin plasma (n=35)	18.7	5.61-77.9	13.0
Citrate plasma (n=25)	13.8	2.66-69.3	13.1

Cell Culture Supernates:
Human peripheral blood lymphocytes (1 x 10^6 cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1 and 6 days. Aliquots of the cell culture supernates were removed and assayed for levels of human Total Serpin E1.

Condition	Day 1 (ng/mL)	Day 6 (ng/mL)
Unstimulated	ND	1.20
Stimulated	ND	ND

ND=Non-detectable

IMR-90 human lung fibroblast cells were cultured overnight in MEM containing NEAA, Earle's salts, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for human Serpin E1, and measured 144 ng/mL.

HUVEC human umbilical vein endothelial cells were grown for 1 day in EGM-2 media to 80% confluency. An aliquot of the cell culture supernate was removed, assayed for human Serpin E1, and measured 174 ng/mL.

HepG2 human hepatocelluar carcinoma cells were grown for 4 days in MEM with NEAA, Earle's salts, 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for human Serpin E1, and measured 42.8 ng/mL.

U2OS human osteocarcinoma cells were cultured overnight in McCoy's 5a media supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for human Serpin E1, and measured 27.8 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Total Serpin E1 (active, latent, uPA, tPA, and Vitronectin complexed Serpin E1).

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 Total Serpin E1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Serpin A1
Serpin A3
Serpin A4
Serpin A5
Serpin A11
Serpin B5
Serpin B6
Serpin B8
Serpin B9
Serpin C1
Serpin D1
Serpin E2
Serpin F2
Serpin I1
Serpin I2
tPA
uPA
uPAR
Vitronectin

Recombinant mouse:

Serpin C1
Serpin E2
Vitronectin
uPAR

Recombinant bovine:

Vitronectin

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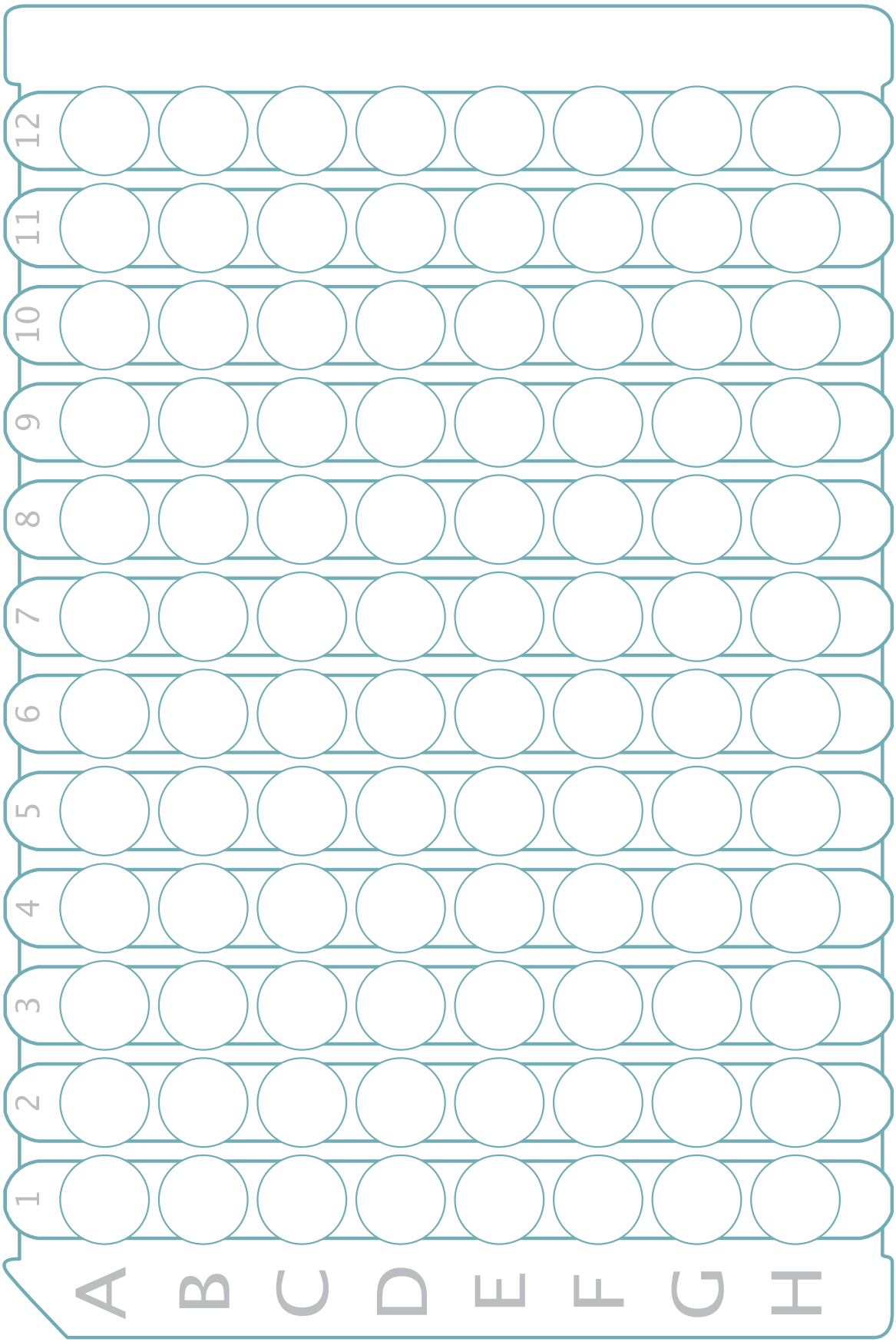
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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