Quantikine® ELISA

Human Serpin A12/Vaspin Immunoassay

Catalog Number DSA120

For the quantitative determination of human Serpin A12 concentrations in cell culture supernates, serum, plasma, saliva, urine, and human milk.

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 A12, also known as Vaspin, is a 45-50 kDa secreted adipokine that contributes to the maintenance of insulin sensitivity (1, 2). It is structurally related to the Serpin family of serine protease inhibitors (3). Mature human Serpin A12 shares 61% amino acid sequence identity with mouse and rat Serpin A12 (3). It is expressed by adipocytes in visceral and subcutaneous fat as well as in the gastric glands and epithelium, and in the placenta (3-5). Serpin A12 administration improves glucose tolerance and insulin sensitivity in obese rats, and it protects vascular endothelial cells from free fatty acid-induced apoptosis (3, 6). Circulating levels of Serpin A12 are higher in women compared to men (7, 8). It is elevated in male but not in female patients with metabolic syndrome, although it is elevated in women with calcified coronary artery stenosis (9). Serum Serpin A12 levels have been shown to be variably associated with insulin resistance, high body mass index (BMI), renal function, liver fibrosis, and HDL cholesterol (8, 10-12). It is decreased by insulin infusion and fasting and is increased by long term physical training (7, 13, 14).

The Quantikine Human Serpin A12/Vaspin Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Serpin A12 in cell culture supernates, serum, plasma, saliva, urine, and human milk. It contains NS0-expressed recombinant human Serpin A12 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Serpin A12 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 A12.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Serpin A12 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Serpin A12 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Serpin A12 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 A12 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, 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 Serpin A12 Microplate	894725	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Serpin A12.	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 Serpin A12 Standard	894727	2 vials of recombinant human Serpin A12 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a new Standard for each assay.
Human Serpin A12 Conjugate	894726	21 mL of a polyclonal antibody specific for human Serpin A12 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-9	895167	11 mL of a buffered protein base with preservatives. *May contain a precipitate. Warm to room temperature and mix gently to dissolve. If the precipitate does not completely dissolve, mix well during use.*	
Calibrator Diluent RD5-54	895598	21 mL 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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards.
- Human Serpin A12 Controls (optional; available from R&D Systems).

PRECAUTIONS

Serpin A12 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 ProClin® 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.*
Grossly hemolyzed samples are not suitable for use in this assay.

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.

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.

Human Milk - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

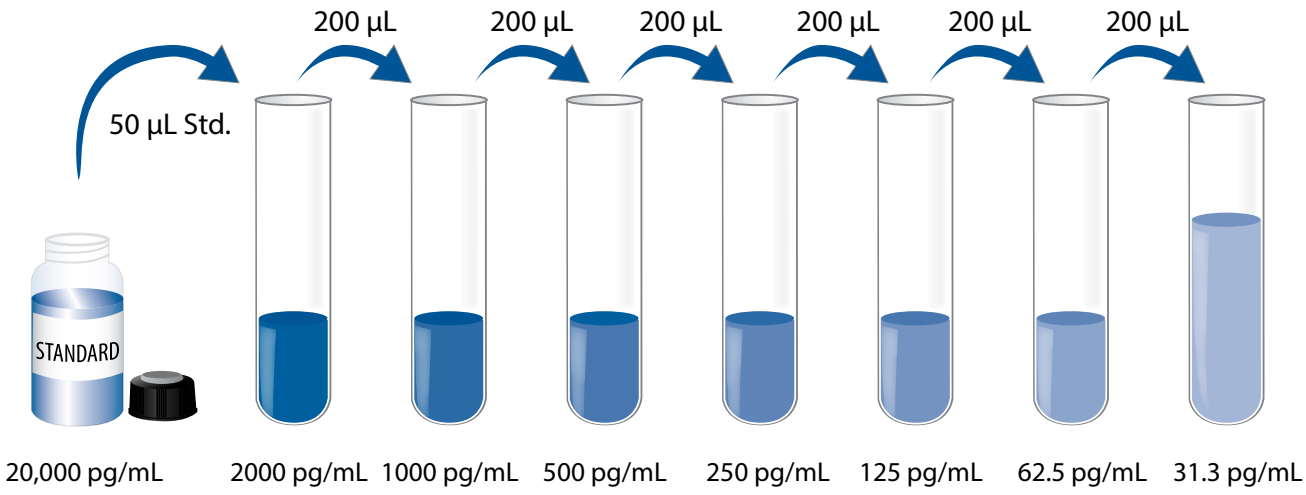
Note: *Serpin A12 is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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 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 Serpin A12 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Serpin A12 Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/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.

Use polypropylene tubes. Pipette 450 µL of Calibrator Diluent RD5-54 into the 2000 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-54 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 samples, controls, and standards be assayed in duplicate.

Note: *Serpin A12 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 RD1-9 to each well. *Assay Diluent RD1-9 may contain a precipitate. Warm to room temperature and mix gently to dissolve. If the precipitate does not completely dissolve, mix well during use.*
4. Add 50 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 Serpin A12 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours 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.

CALCULATION OF RESULTS

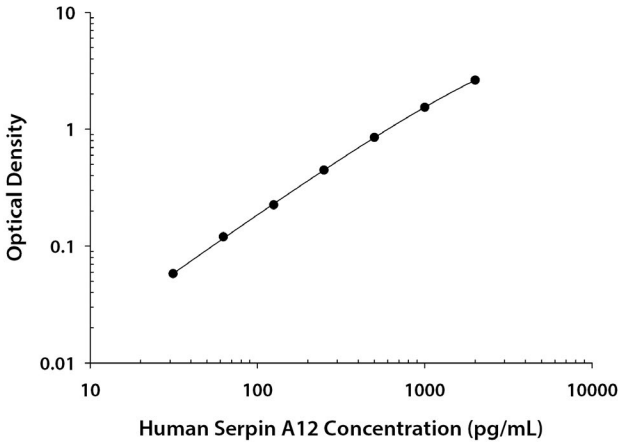
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 Serpin A12 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.



(pg/mL)	O.D.	Average	Corrected
0	0.023 0.025	0.024	—
31.3	0.081 0.083	0.082	0.058
62.5	0.142 0.146	0.144	0.120
125	0.242 0.255	0.249	0.225
250	0.458 0.483	0.471	0.447
500	0.866 0.886	0.876	0.852
1000	1.557 1.573	1.565	1.541
2000	2.636 2.661	2.649	2.625

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)	170	531	1061	173	523	1033
Standard deviation	8.57	10.6	39.9	11.2	30.6	66.6
CV (%)	5.0	2.0	3.8	6.5	5.9	6.4

RECOVERY

The recovery of human Serpin A12 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	98	92-107%
Serum (n=4)	92	85-99%
EDTA plasma (n=4)	90	80-97%
Heparin plasma (n=4)	92	84-98%
Urine (n=4)	93	83-100%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Serpin A12 were 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)	Saliva (n=4)	Urine (n=4)	Human milk (n=4)
1:2	Average % of Expected	103	99	99	101	106	103	99
	Range (%)	97-108	97-102	96-104	98-107	101-109	100-106	89-107
1:4	Average % of Expected	97	103	105	103	106	104	108
	Range (%)	95-100	100-105	102-107	98-114	100-109	100-106	104-112
1:8	Average % of Expected	101	106	106	105	105	105	117
	Range (%)	95-109	101-114	97-115	96-116	97-116	100-111	116-117
1:16	Average % of Expected	100	102	101	98	103	105	113
	Range (%)	93-106	95-106	95-106	95-106	95-109	102-108	———

SENSITIVITY

Thirty-four assays were evaluated and the minimum detectable dose (MDD) of human Serpin A12 ranged from 0.772-14.6 pg/mL. The mean MDD was 4.66 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 NS0-expressed recombinant human Serpin A12 manufactured at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of human Serpin A12 in this assay. No medical histories were available for the donors used in this study.

Male Samples	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=17)	123	88	ND-256
EDTA plasma (n=17)	123	82	ND-231
Heparin plasma (n=17)	114	100	35.4-246

Female Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=17)	273	81.8-811	206
EDTA plasma (n=17)	274	71.9-822	211
Heparin plasma (n=17)	267	73.9-819	203

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Saliva (n=12)	385	92	ND-1240
Urine (n=10)	209	30	ND-459
Human milk (n=18)	183	61	ND-734

ND=Non-detectable

Outlying samples were removed from the above serum/plasma tables.

Outlying Samples	Serum (pg/mL)	EDTA plasma (pg/mL)	Heparin plasma (pg/mL)
Male (n=1)	25,608	26,075	27,308
Female (n=1)	5568	5509	5715

Cell Culture Supernates - Seventy-nine primary cells and cell lines were tested for human Serpin A12. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant human Serpin A12.

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 Serpin A12 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

GRK7
Serpin A1
Serpin A2
Serpin A3
Serpin A4
Serpin A5
Serpin A6
Serpin A7
Serpin A8
Serpin A9
Serpin A10
Serpin A11
Serpin B2
Serpin B3
Serpin B5
Serpin B8
Serpin B9
Serpin C1
Serpin D1
Serpin E1
Serpin E2
Serpin F1
Serpin F2
Serpin G1
Serpin I1
Thrombin
Trypsin 3

Recombinant mouse:

Serpin A1
Serpin A1A
Serpin A3N
Serpin A5
Serpin A6
Serpin A8
Serpin A9
Serpin A11
Serpin B2
Serpin D1
Serpin E1
Serpin E2
Serpin F1
Serpin F2
Serpin I1

Recombinant rat:

Serpin A6
Serpin A12

Natural proteins:

Prothrombin

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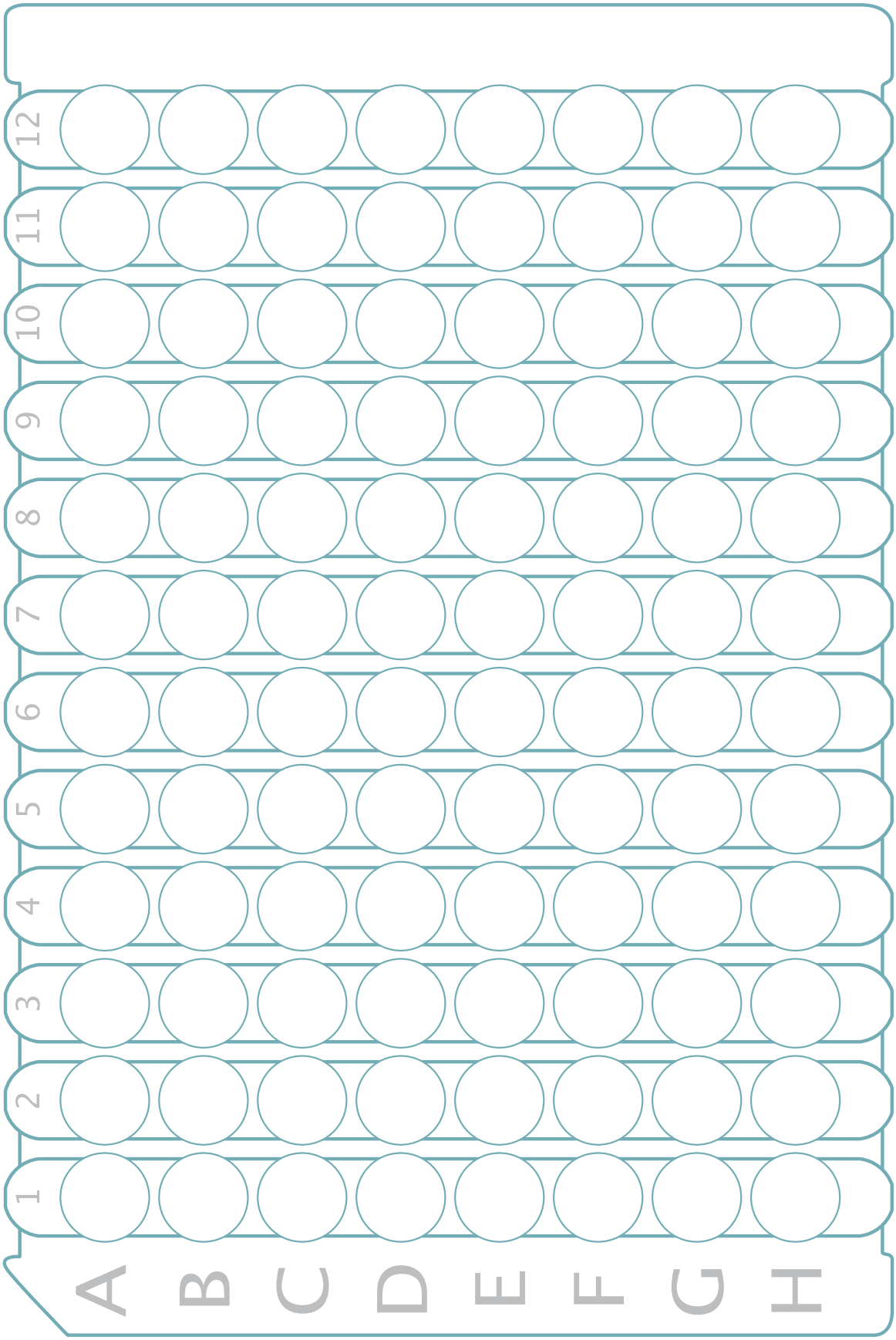
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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