Quantikine™ ELISA

Human Serpin G1/C1 Inhibitor Immunoassay

Catalog Number DSGC10

For the quantitative determination of human Serpin G1 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 G1, also known as C1-Esterase Inhibitor (C1Inh) is a well described (1), heavily glycosylated serine protease inhibitor. It is a member of the serpin superfamily and regulates processes of vascular homeostasis, including inflammation, blood pressure, vascular permeability and coagulation. Observed Serpin G1 molecular weight species range from 100 kilodalton (kDa) in cell lysates to 80 kDa in cell culture media, and 68 kDa or 71 kDa after deglycosylation by endoglycosidase F or endoglycosidase H respectively. The mature protein contains 500 amino acid residues and is divided into a two domain structure. The N-terminal domain contains most of the glycosylation sites. It is not essential for Serpin G1's protease inhibitory activity and is highly divergent across species. The C-terminal globular serpin domain is responsible for Serpin's inhibitory activity. Binding to proteases involves cleavage of Serpin G1, formation of a covalent bond with the protease and the induction of significant conformational changes to the active site of the protease. Proteases that are inactivated by Serpin G1 include C1r and C1s (classical pathway of complement), mannan binding lectin-associated serine protease (MASP) 1 and 2 (lectin pathway), coagulation factor XII and plasma kallikrein (contact system), coagulation factor XI and thrombin (coagulation system) and plasmin and tissue plasminogen activator (fibrinolytic system).

Although Serpin G1 is primarily synthesized by hepatocytes, it is also expressed in cells of the monocyte/macrophage lineage. Sites of Serpin G1 synthesis include macrophages, microglia, fibroblasts, endothelial cells, placenta and megakaryocytes. Serpin G1 has been observed in several other organs including, brain, spleen, heart kidney and lung. Extrahepatic Serpin G1 is typically induced by inflammation or tissue injury, resulting in a 2.5 fold increase in serum levels. Several cytokines stimulate Serpin G1, including interferon gamma (IFN-γ), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α). As alluded to previously, Serpin G1 controls vascular permeability via the inhibition of coagulation factor XIIa and plasma kallikrein. These two proteases are important for the generation of bradykinin (BK), which increases vascular permeability. Serpin G1's anti-inflammatory effects occur via a variety of mechanisms, including inhibition of complement C1s, MASP2, plasma contact system proteases, interactions with leucocytes as well as with endothelial cells via E and P Selectins (2).

Hereditary Angioedema (HAE) is an autosomal dominant disease caused by mutation of the Serpin G1 gene located on chromosome 11 (3). At least 275 Serpin mutations have been identified. Twenty five percent of the HAE mutations are spontaneous. HAE symptoms are characterized by recurrent acute subcutaneous edema as well as swelling in areas such as the gastrointestinal tract, larynx or bowels due to increased vascular permeability (1,3). Lack of Serpin G1 results in excessive bradykinin expression via unchecked coagulation factor XIIa. Two forms of HAE are known, Type 1, and Type 2 (3). Type 1 HAE accounts for 85% of cases and is a result of decreased Serpin G1 production. The remaining 15% of cases are Type 2, which is characterized by normal or elevated levels of a non-functional Serpin G1.

The Quantikine™ Human Serpin G1/C1 Inhibitor Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Serpin G1 in cell culture supernates, serum, plasma, saliva, urine, and human milk. It contains human Serpin G1 purified from plasma and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Serpin G1 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 Serpin G1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Serpin G1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Serpin G1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Serpin G1 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 G1 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 Serpin G1 Microplate	899240	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Serpin G1.	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 G1 Standard	899242	2 vials of human Serpin G1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* ***Note: Human sourced material. See Precaution section.***	Use a new standard for each assay. Discard after use.
Human Serpin G1 Conjugate	899241	21 mL of a polyclonal antibody specific for human Serpin G1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-55	895066	11 mL of a buffered protein base with blue dye and preservatives.	
Calibrator Diluent RD5P	895151	2 vials (21 mL/vial) of a buffered protein base with preservatives. *Use diluted 1:5 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
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- 100 mL and 500 mL graduated cylinders
- Test tubes for dilution of standards and samples
- Human Serpin G1 Controls (optional; R&D Systems®, Catalog # QC288)

PRECAUTIONS

The Serpin G1 standard provided with this kit was prepared from human serum or plasma. The source material was tested at the donor level using FDA licensed methods and found to be non-reactive for anti-HIV-1/2 and Hepatitis B surface antigen. As no testing can offer complete assurance of freedom from infectious agents, this reagent should be handled as if capable of transmitting infection.

The Stop Solution provided with this kit is an acid solution.

Serpin G1 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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 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.*

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.

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.

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.

SAMPLE PREPARATION

Cell culture supernate samples may require a dilution due to high endogenous values.

Serum and plasma samples require a 100,000-fold dilution due to high endogenous values. A suggested 100,000-fold dilution can be achieved by adding 10 µL of sample to 490 µL of Calibrator Diluent RD5P (diluted 1:5)*. Then add 10 µL of the diluted sample to 490 µL Calibrator Diluent RD5P (diluted 1:5)*. Complete the 100,000-fold dilution by adding 10 µL of the diluted sample to 390 µL Calibrator Diluent RD5P (diluted 1:5)*.

Saliva and urine samples require a 100-fold dilution due to high endogenous values. A suggested 100-fold dilution can be achieved by adding 10 µL of sample to 990 µL of Calibrator Diluent RD5P (diluted 1:5)*.

Human milk samples require a 700-fold dilution due to high endogenous values. A suggested 700-fold dilution can be achieved by adding 10 µL of sample to 990 µL of Calibrator Diluent RD5P (diluted 1:5)*. Complete the 700-fold dilution by adding 70 µL of the diluted sample to 420 µL Calibrator Diluent RD5P (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *Serpin G1 is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

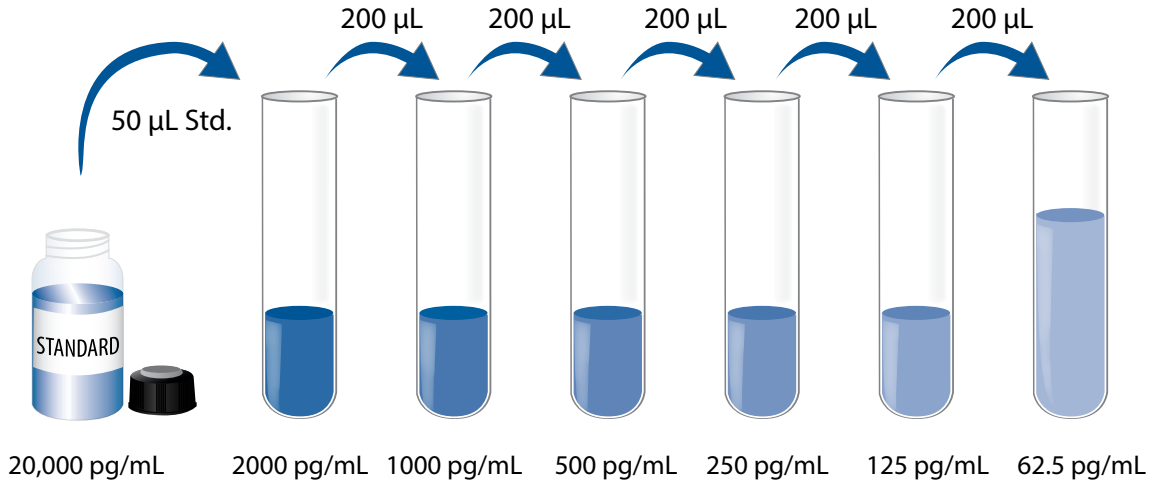
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Human Serpin G1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Serpin G1 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 5 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5P (diluted 1:5) 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 RD5P (diluted 1:5) 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, controls, and samples be assayed in duplicate.

Note: *Serpin G1 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 50 µL of Assay Diluent RD1-55 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 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 G1 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.

*Samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

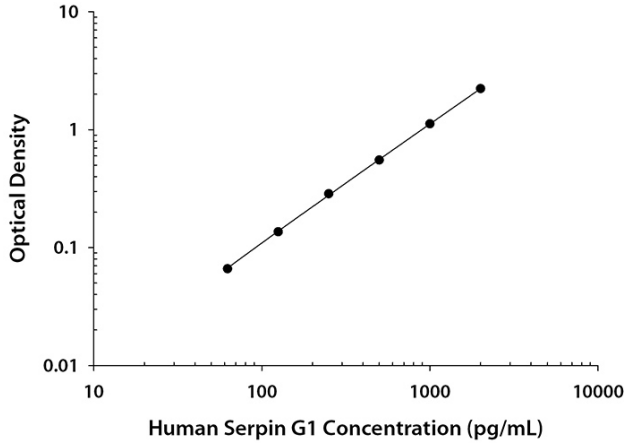
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 Serpin G1 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	0.026 0.039	0.033	—
62.5	0.096 0.102	0.099	0.066
125	0.166 0.172	0.169	0.136
250	0.307 0.330	0.319	0.286
500	0.579 0.592	0.586	0.553
1000	1.128 1.180	1.154	1.121
2000	2.235 2.289	2.262	2.229

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)	206	634	1209	212	603	1191
Standard deviation	10.4	24.7	56.5	13.7	31.7	53.4
CV (%)	5.0	3.9	4.7	6.5	5.3	4.5

RECOVERY

The recovery of human Serpin G1 spiked to levels throughout the range of the assay in cell culture media was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	100	90-110%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Serpin G1 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. Sample were diluted prior to assay.

		Cell culture media (n=4)	Cell culture supernates (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	98	97	97	104	96	99	108
	Range (%)	100-105	97-101	95-100	90-105	99-107	90-100	89-106	98-117
1:4	Average % of Expected	108	99	95	96	101	103	95	108
	Range (%)	106-111	97-101	84-100	88-102	97-104	91-121	81-101	96-117
1:8	Average % of Expected	110	98	94	95	105	99	92	108
	Range (%)	107-112	94-103	91-98	77-105	102-110	92-106	78-104	92-121
1:16	Average % of Expected	105	97	91	104	100	92	92	102
	Range (%)	102-109	96-98	91-91	104-104	100-100	87-95	76-104	87-110

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

SENSITIVITY

Thirty-two assays were evaluated and the minimum detectable dose (MDD) of human Serpin G1 ranged from 2.01-25.6 pg/mL. The mean MDD was 9.01 pg/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 highly purified Serpin G1 from human plasma produced at R&D Systems®.

The NIBSC/WHO Serpin C1-Inhibitor International Standard 08/256 was evaluated in this kit. The dose response curve of the reference reagent 08/256 parallels the Quantikine™ standard curve. To convert sample values obtained with the Quantikine Human Serpin G1/C1 Inhibitor kit to approximate NIBSC/WHO 08/256 Units, use the equation below:

NIBSC/WHO (08/256) approximate value (µIU/mL) = 0.0117 x Quantikine Human Serpin G1/C1 Inhibitor value (pg/mL)

Note: *Based on data generated in August 2021.*

SAMPLE VALUES

Serum/Plasma/Saliva/Urine/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of human Serpin G1 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=30)	138,746	55,639-323,345	56,643
EDTA plasma (n=30)	136,446	76,442-259,277	39,166
Heparin plasma (n=30)	131,218	77,947-225,264	37,971
Saliva (n=10)	50.5	11.0-121	35.8
Urine (n=10)	66.1	16.9-176	56.8
Human milk (n=10)	601	238-1318	364

Cell Culture Supernates:
Unstimulated THP-1 human acute monocytic leukemia cells were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until they reached a cell density of approximately 1x10 ^{6} cells/mL. An aliquot of the cell culture supernate was removed, assayed for human Serpin G1, and measured less than the lowest standard, 62.5 pg/mL.

Stimulated THP-1 cells were grown in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and 50 µM β-mercaptoethanol to a density of 1 x 10^{6} cells/mL. Cells were differentiated into macrophages with 150 nM PMA for 72 hours and then treated with 100 ng/mL recombinant human IFN-γ (R&S Systems, Catalog # 285-IF) for 48 hours before collecting conditioned media. An aliquot of the cell culture supernate was removed, assayed for human Serpin G1, and measured at 62.4 ng/mL.

SPECIFICITY

This assay recognizes natural human Serpin G1.

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

Recombinant human:

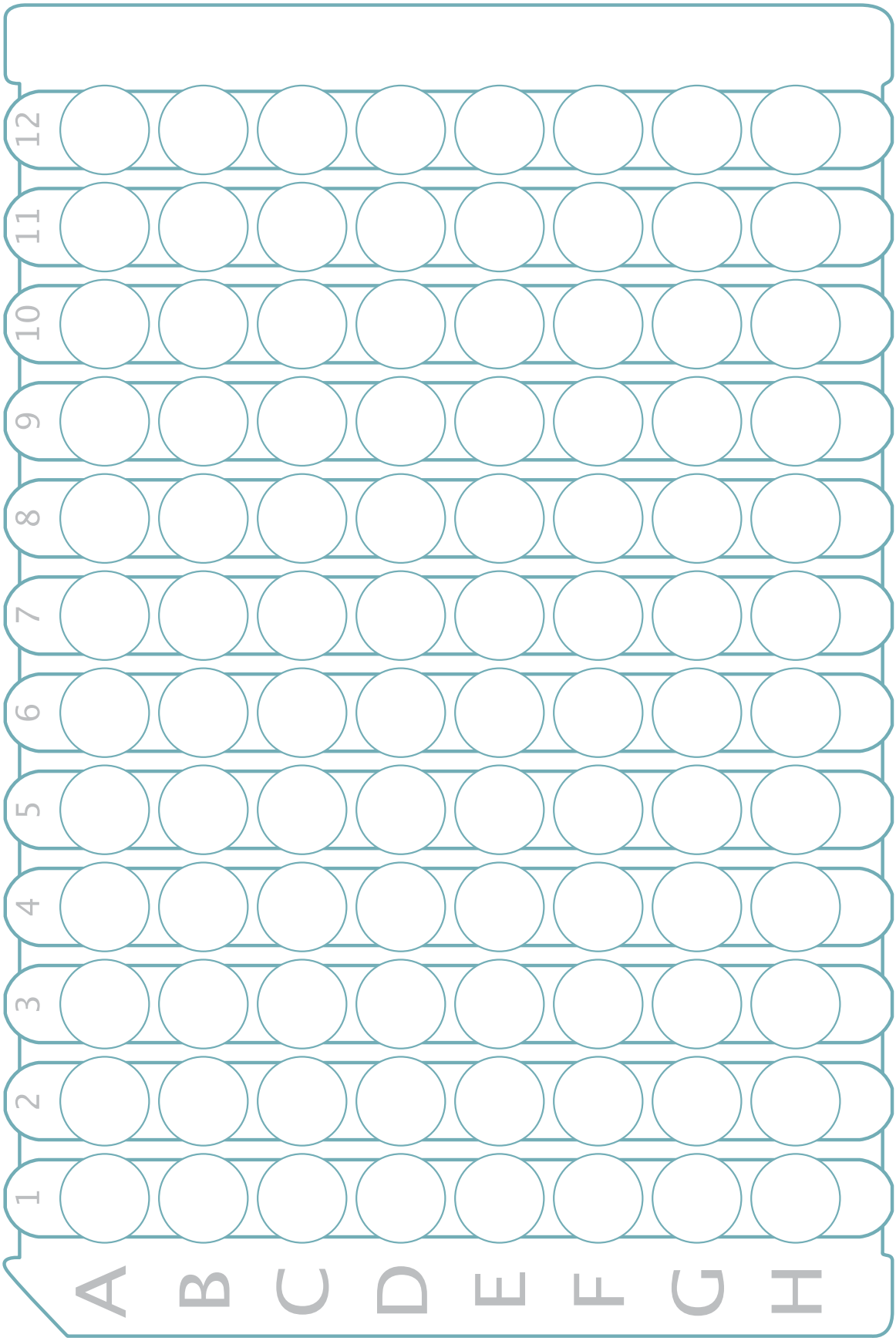
C1R
C1s
Factor XI
Plasminogen
PLAT
Serpin A1
Serpin A3
Serpin B9
Serpin F1
Serpin F2
Thrombin

REFERENCES

1. Drouet, C. *et al.* (2018) The Complement Facts Book. Ch. 23
2. Davis, A.E. *et al.* (2010) Thromb Haemost. **104**:886
3. Gower, R. G. *et al.* (2011) WAO Journal. **4**:S9

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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