Quantikine® ELISA

Human Serpin E1/PAI-1 Immunoassay

Catalog Number DSE100

For the quantitative determination of human Serpin E1/PAI-1 concentrations in cell culture supernates, cell lysates, 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.

TABLE OF CONTENTS

MANUFACTURED AND DISTRIBUTED BY:

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

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Serpin E1, also known as Plasminogen Activator Inhibitor-1 (PAI-1), is a member of the serpin superfamily of serine protease inhibitors (1). 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, fibrosis, angiogenesis, wound healing, and tumor cell invasion and metastasis (2). For example, high levels of uPA and Serpin E1 correlate with poor prognosis in patients with breast cancer, and uPA and Serpin E1 are among the most reliable biomarkers whose clinical values were confirmed in level 1 evidence (LOE-1) studies (3). Besides breast cancer, Serpin E1 has been linked to many diseases such as metabolic syndrome, asthma, major depressive disorder (MDD), and aging-related pathologies (4-8). The absence of Serpin E1 in plasma causes abnormal bleeding, and high plasma levels of Serpin E1 are associated with inherited thrombophilia (9, 10).

The deduced amino acid sequence of human Serpin E1 is 99.5%, 86.1%, 80.8%, and 78.6% identical to that of chimpanzee, canine, rat, and mouse, respectively. Synthesized and secreted by many cell types, free Serpin E1 is relatively unstable in its active form and readily converts into a latent, inactive form by spontaneous insertion of its reactive center loop (RCL) into the β-sheet core of the protein (1, 9). Binding of vitronectin to Serpin E1, however, stabilizes the active form by preventing the conformation change and also broadens the target specificity of Serpin E1 beyond uPA and tPA (1, 11). The current model for the Serpin E1- vitronectin complexes involves the formation of a 2:1 intermediate and a few higher order complexes (11). The active form of Serpin E1 binds tightly with uPA and tPA in a 1:1 ratio. After the formation of an initial docking (Michaelis) complex, the proteases cleave the RCL of Serpin E1 to form a stable, covalent complex, resulting in the inactivation of the targeted protease (1).

The Quantikine Human Serpin E1/PAI-1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Serpin E1 in cell culture supernates, cell lysates, and plasma. It contains *Sf* 21-expressed recombinant human Serpin E1 and has been shown to accurately quantitate the recombinant factor in its active and latent forms as well as in the vitronectin complexes. The kit does not detect recombinant human Serpin E1 in complexes with recombinant human uPA and recombinant human tPA. 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 monoclonal antibody specific for human 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 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.
- Samples, controls, and standards must be pipetted within 15 minutes.
- 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 E1/PAI-1 Microplate	893056	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human 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 Serpin E1/PAI-1 Conjugate	893057	21 mL of polyclonal antibody specific for human Serpin E1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Serpin E1/PAI-1 Standard	893058	200 ng of recombinant human Serpin E1 in a buffered protein solution with preservatives; lyophilized.	
Assay Diluent RD1-57	895207	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-17	895512	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.
- Test tubes for dilution of standards.
- Human Serpin E1/PAI-1 Controls (optional; available from R&D Systems).

OTHER SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Cell Lysis Buffer 3 Concentrate (R&D Systems, Catalog # 895366).
- PBS

PRECAUTIONS

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.

Cell Lysates - Cells must be lysed prior to assay as directed in the Cell Lysis Procedure.

Plasma - Collect plasma using EDTA, heparin, citrate, or CTAD 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: *Hemolyzed or lipemic samples are not suitable for use in this assay.*

CELL LYSIS PROCEDURE

Use the following procedure for the preparation of cell lysate samples.

1. Perform a 5-fold dilution of Cell Lysis Buffer 3 with deionized or distilled water.
2. Wash cells one time in cold PBS.
3. Resuspend cells at 1-5 x 10^6 cells/mL in diluted Cell Lysis Buffer 3.
4. Incubate with gentle agitation for 30 minutes at room temperature and freeze/thaw cells once at ≤ -20 °C.
5. Centrifuge to remove cell debris.
6. Assay immediately or aliquot the lysis supernates and store at ≤ -20 °C until ready for use.

REAGENT PREPARATION

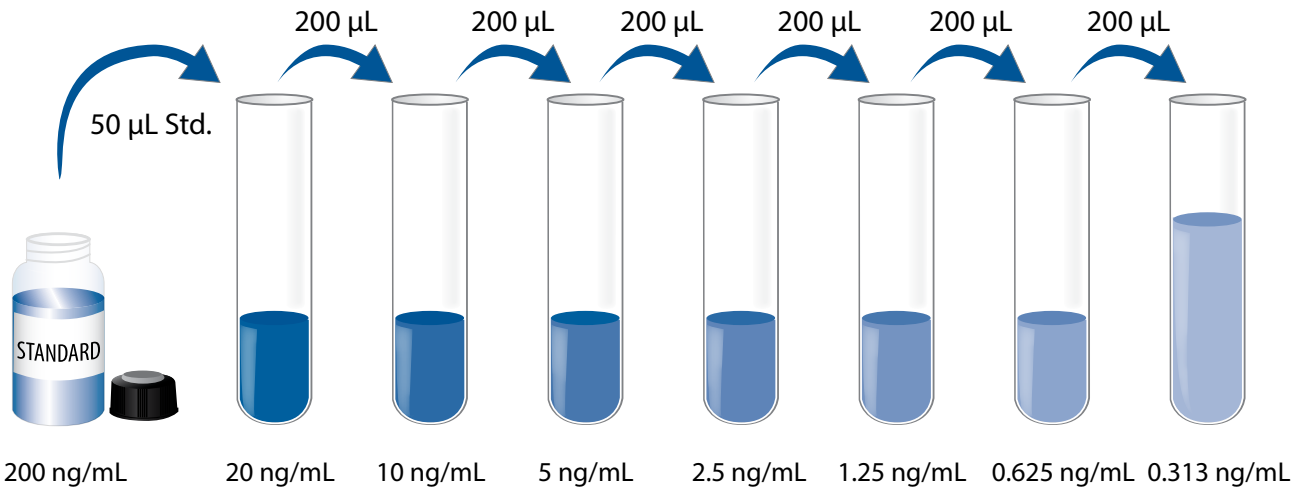
Bring all reagents to room temperature before use.

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 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 E1/PAI-1 Standard - Reconstitute the Human Serpin E1/PAI-1 Standard with 1.0 mL of 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 RD5-17 into the 20 ng/mL tube. Pipette 200 µL of Calibrator Diluent RD5-17 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. The Calibrator Diluent 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 samples, controls, and standards 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. **Ensure that addition to the plate is uninterrupted and completed within 15 minutes.** 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 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.

CALCULATION OF RESULTS

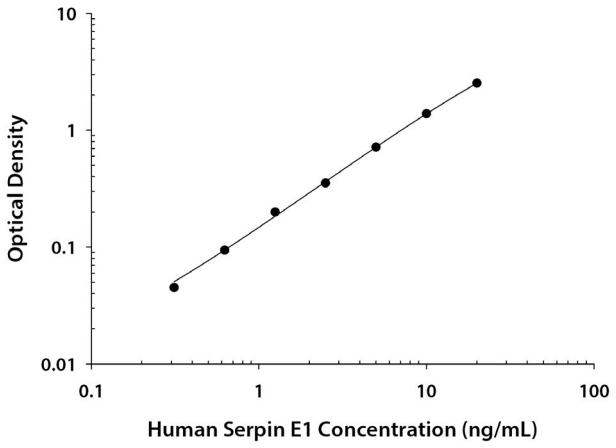
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 E1/PAI-1 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	0.039 0.041	0.040	—
0.313	0.081 0.088	0.085	0.045
0.625	0.131 0.136	0.134	0.094
1.25	0.235 0.242	0.239	0.199
2.5	0.380 0.405	0.393	0.353
5	0.753 0.761	0.757	0.717
10	1.406 1.440	1.423	1.383
20	2.561 2.563	2.562	2.522

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)	2.6	6.5	12.6	2.3	6.4	13.1
Standard deviation	0.12	0.52	0.98	0.20	0.40	0.80
CV (%)	4.6	8.0	7.8	8.7	6.3	6.1

RECOVERY

The recovery of human Serpin E1/PAI-1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	106	89-113%
Citrate plasma (n=4)	99	88-112%
EDTA plasma (n=4)	100	85-115%
Heparin plasma (n=4)	98	85-113%
CTAD plasma (n=4)	100	83-112%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Serpin E1/PAI-1 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=8)	Cell lysates (n=4)	EDTA plasma (n=8)	Heparin plasma (n=8)	Citrate plasma (n=8)	CTAD plasma (n=8)
1:2	Average % of Expected	95	96	102	100	102	101
	Range (%)	87-107	95-97	94-109	86-111	90-110	88-114
1:4	Average % of Expected	92	98	99	101	99	101
	Range (%)	85-100	92-104	91-111	87-113	90-113	88-115
1:8	Average % of Expected	92	95	100	101	100	100
	Range (%)	87-97	92-99	89-114	93-109	91-108	89-114
1:16	Average % of Expected	92	85	100	96	98	98
	Range (%)	87-96	———	86-113	85-106	86-111	88-113

SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of human Serpin E1 ranged from 0.014-0.142 ng/mL. The mean MDD was 0.059 ng/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 *Sf* 21-expressed recombinant human Serpin E1 produced at R&D Systems.

SAMPLE VALUES

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)
EDTA plasma (n=35)	5.10	0.99-16.9	3.74
Heparin plasma (n=35)	4.57	0.98-18.7	3.67
Citrate plasma* (n=12)	4.23	0.74-14.0	4.08
CTAD plasma* (n=9)	4.43	1.19-8.50	2.71

*Values can be affected by variability in collection, processing, and sample dilution due to the anticoagulant in the tubes.

Cell Culture Supernates:

Human peripheral blood lymphocytes (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal calf 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 for 1 and 6 days. Aliquots of the cell culture supernates were removed and assayed for levels of human Serpin E1.

Condition	Day 1 (ng/mL)	Day 6 (ng/mL)
Unstimulated	ND	ND
Stimulated	ND	0.779

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 43.6 ng/mL.

HUVEC human umbilical vein endothelial cells were grown for 4 days in EGM media containing 2% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and bovine brain extracts. An aliquot of the cell culture supernate was removed, assayed for human Serpin E1, and measured 39.6 ng/mL.

SAMPLE VALUES *CONTINUED*

HepG2 human hepatocelluar carcinoma cells were grown for 4 days in MEM with NEAA, Earle's salts, 10% fetal bovine serum, 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 14.9 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 1.71 ng/mL.

Cell Lysates:
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. The cell culture supernate was removed, and the cells were resuspended at 5×10^6 cells/mL in diluted Cell Lysis Buffer 3. After one freeze/thaw, the lysate was assayed for human Serpin E1, and measured 26.6 ng/mL.

HUVEC human umbilical vein endothelial cells were grown for 4 days in EGM media containing 2% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and bovine brain extracts. The cell culture supernate was removed, and the cells were resuspended at 1×10^6 cells/mL in diluted Cell Lysis Buffer 3. The lysate was assayed for human Serpin E1, and measured 20.2 ng/mL.

HMVEC human microvascular endothelial cells were grown to 80% confluency in EGM media containing 2% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and bovine brain extracts. The cell culture supernate was removed, and the cells were resuspended at 1×10^6 cells/mL in diluted Cell Lysis Buffer 3. The lysate was assayed for human Serpin E1, and measured 29.7 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human 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 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 B6
Serpin B8
Serpin B9
Serpin C1
Serpin D1
Serpin F2
Serpin I1
Serpin I2
uPAR
Vitronectin

Recombinant mouse:

Serpin E1
Vitronectin

Recombinant human Serpin E2 cross-reacts at concentrations > 100 ng/mL.

Human tPA and uPA interfere when adding more than 10 ng/mL to an EDTA plasma pool containing endogenous tPA and uPA.

Note: *Normal levels of tPA and uPA in plasma are approximately 10 ng/mL and 1 ng/mL respectively.*

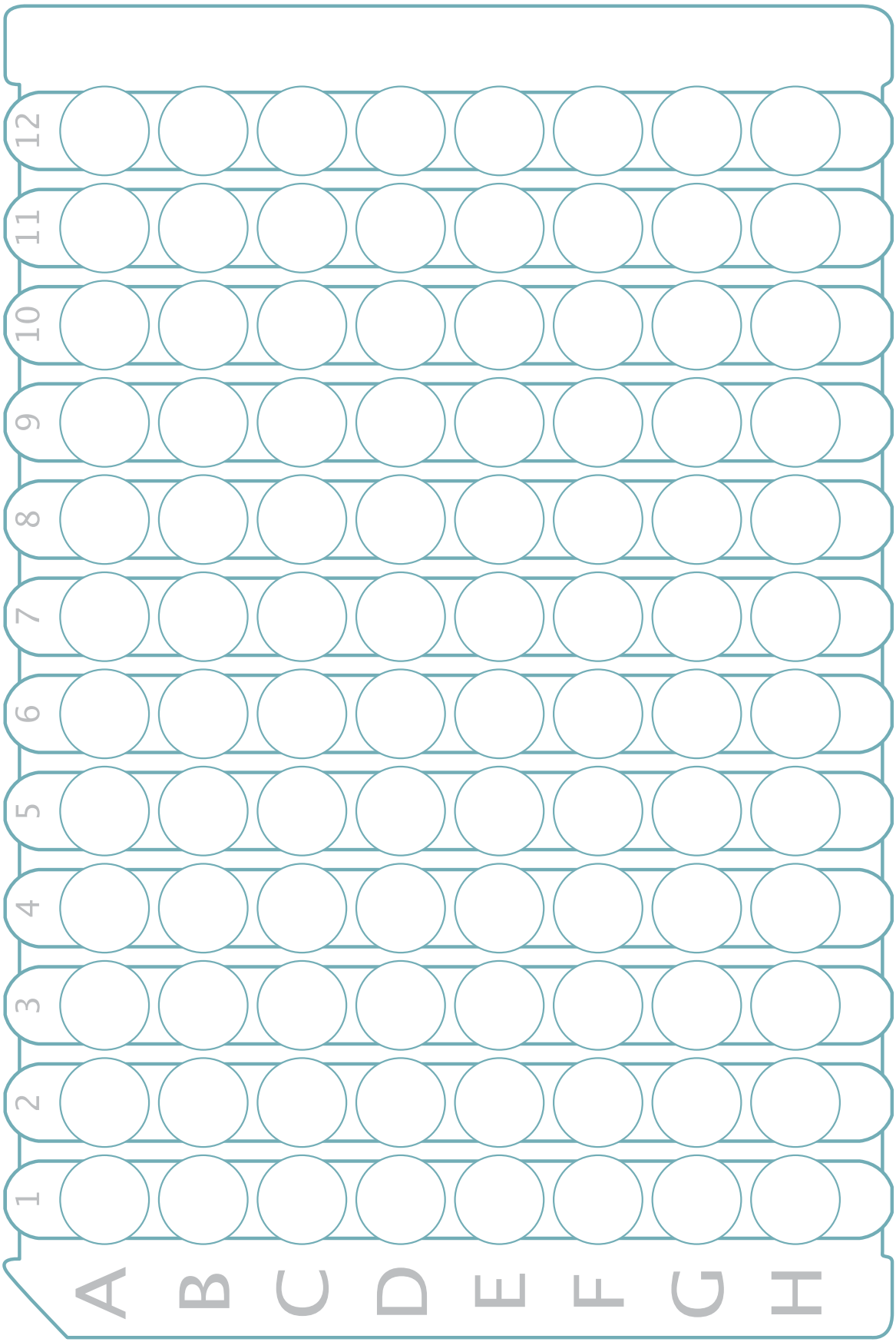
The active and latent forms of recombinant human Serpin E1 and the complexes formed between the active form and recombinant human uPA, tPA, and vitronectin were tested. The kit detected the active and latent forms of recombinant human Serpin E1 and Serpin E1 in the vitronectin complex. The kit did not detect Serpin E1 in the uPA and tPA complexes.

REFERENCES

1. Law, R.H.P. *et al.* (2006) Genome Biol. **7**:216.
2. Heaton, J.H. *et al.* (2003) Thromb. Haemost. **89**:959.
3. Duffy, M.J. (2002) Clin. Chem. **48**:1194.
4. Han, B. *et al.* (2005) Oncol. Rep. **14**:105.
5. Alessi, M.C. and I. Juhan-Vague (2006) Arterioscler. Thromb. Vasc. Biol. **26**:2200.
6. Cho, S.H. *et al.* (2004) Exp. Biol. Med. **229**:138.
7. Tsai, S.J. (2006) Med. Hypotheses **66**:319.
8. Yamamoto, K. *et al.* (2005) Cardiovasc. Res. **66**:276.
9. Fay, W.P. *et al.* (1997) Blood **90**:204.
10. Sartori, M.T. *et al.* (2003) Clin. Appl. Thromb. Hemost. **9**:299.
11. Minor, K.H. *et al.* (2005) J. Biol. Chem. **280**:28711.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES