Human SERPINE1 Sandwich ELISA Kit Datasheet

Catalogue Number: KE00109

Size: 5*96T

Sensitivity: 0.16 ng/mL

Range: 0.312-20 ng/mL

Usage: For the quantitative detection of human SERPINE1 concentrations in serum, plasma, cell culture supernatant and cell lysate.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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1. Background

SERPINE1, also named as Plasminogen activator inhibitor type 1 (PAI-1), is a member of the serine protease inhibitor (SERPIN) superfamily. It is produced by the vascular endothelium, the liver, the monocytes/macrophagues, the platelets and the adipose tissue. High plasma levels of PAI-1 have been associated with an increased risk of suffering cardiovascular diseases. It is implicated in the pathogenesis of obesity, insulin resistance and type 2 diabetes. In several tumor types, SERPINE1 expression is up-regulated and it has been described as a poor prognostic marker. Besides its prognostic value, SERPINE1 expression has been validated as a marker for therapy decision making in patients with node-negative breast cancer. Defects in this gene are the cause of plasminogen activator inhibitor-1 deficiency (PAI-1 deficiency), and high concentrations of the gene product are associated with thrombophilia.

2. Principle

Y	Capture antibody
◯	Target
Y	Detection antibody, HRP-conjugated
ᐁᐁᐁ	TMB
✳	Stop Solution
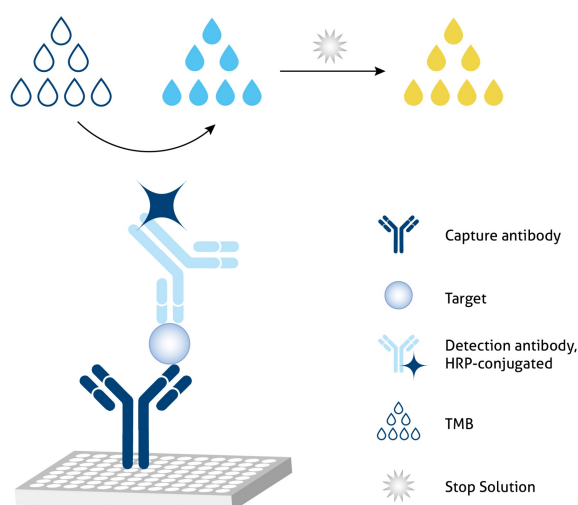
Sandwich ELISA structure (Detection antibody labeled with HRP)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with HRP also binds to the analyte. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	5 plates	**Unopened Kit:**
Protein standard - 20 ng/bottle; lyophilized	10 bottles	
Detection antibody, HRP-conjugated (100×) - 600 µL/vial*	1 vial	Store at 2-8°C for 6 months or -20°C for 12 months.
Sample Diluent PT 4 - 150 mL/bottle. For serum and plasma	1 bottle	
Sample Diluent PT 1 - 150 mL/bottle. For cell culture supernatant and cell lysate	1 bottle	**Opened Kit:**
Detection Diluent - 150 mL/bottle	1 bottle	All reagents stored at 2-8°C for 7 days.
Wash Buffer Concentrate (20×) - 150 mL/bottle	1 bottle	
Extraction Reagent - 150 mL/bottle	1 bottle	**Please use a new standard for each assay.**
Tetramethylbenzidine Substrate (TMB) - 60 mL/bottle	1 bottle	
Stop Solution - 60 mL/bottle	1 bottle	
Plate Cover Seals	15 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20℃. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20℃. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

6.4 Cell Lysate:

1) Collect cells and wash by centrifuging at 500 x g for 5 minutes before resuspension in pre-cooled PBS buffer. Perform this step three times.

2) Count cells and then discard the supernatant.

3) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing cell lysis.

4) Add 1 mL of Extraction reagent (containing protease inhibitor cocktail) Per 1 x 107 cells, Incubate cell suspension on ice for 30 minutes, use ultrasound to treat the samples.

5) Centrifuge cell lysate at 10,000 x g for 5 minutes at 4℃.

6) Measure the concentration of total protein in cell lysate using BCA assay. Where possible, keep samples on ice to avoid protein degradation.

7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.
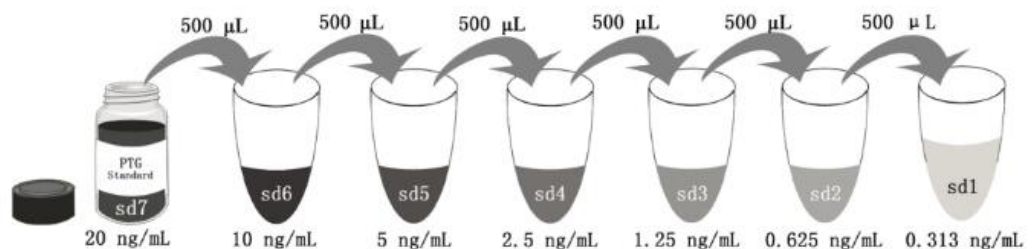
7.2 Detection Antibody, HRP-conjugated (1X): Dilute 100X Detection Antibody, HRP-conjugated 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 μL 100X Detection Antibody, HRP-conjugated + 990 μL Detection Diluent (Centrifuge the 100 X Detection Antibody solution, HRP-conjugated for a few seconds prior to use)

7.3 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:80 or 1:160 is recommended for human serum and plasma; 1:16 or 1:32 is recommended for cell culture supernatant; 1:8 or 1:16 is recommended for cell lysate.

7.4 Standard Serial Dilution:

For human serum and plasma, add 1 mL Sample Diluent PT 4 in protein standard; For cell culture supernatant and cell lysates, add 1 mL Sample Diluent PT 1 in protein standard.

Add # μL of Standard diluted in the previous step	—	500 μL	500 μL	500 μL	500 μL	500 μL	500 μL
# μL of Sample Diluent PT 4 or PT 1	**1000 μL**	500 μL	500 μL	500 μL	500 μL	500 μL	500 μL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated can be used immediately). 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.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4℃ immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group,standard group and sample group, add 100 μL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 1 hours at 37℃.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 μL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 μL of 1X Detection antibody, HRP-conjugated solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 40 minutes at 37℃.

8.6 Repeat wash step in 8.4.

8.7 Signal development: Add 100 μL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.8 Quenching color development: Add 100 μL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.9 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.10 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

| Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 μL	60 min	4 times	Cover Wells incubate at 37℃
2	Diluent Detection antibody, HRP-conjugated Solution	100 μL	40 min	4 times	Cover Wells incubate at 37℃
3	TMB Substrate	100 μL	15-20 min	Do not wash	Incubate in the dark at 37℃
4	Stop Solution	100 μL	0 min	Do not wash	-
5	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Sample Diluent PT 4
Four Parameter Logistic (4-PL) Curve Fit

$R^2=0.999$
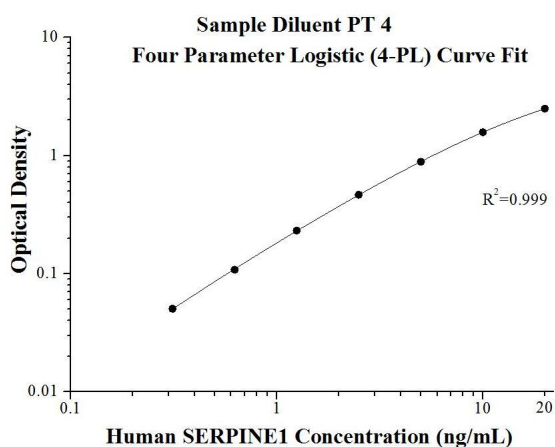
(ng/mL)	O.D	Average	Corrected
0	0.077 0.086	0.081	-
0.313	0.137 0.127	0.132	0.05
0.625	0.192 0.187	0.19	0.108
1.25	0.311 0.315	0.313	0.231
2.5	0.547 0.549	0.548	0.467
5	0.964 0.969	0.967	0.885
10	1.67 1.646	1.658	1.577
20	2.58 2.564	2.572	2.49

Sample Diluent PT 1
Four Parameter Logistic (4-PL) Curve Fit

$R^2=0.999$
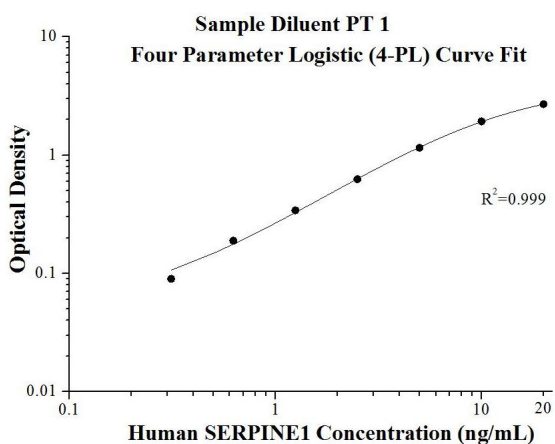
(ng/mL)	O.D	Average	Corrected
0	0.069 0.074	0.072	-
0.313	0.165 0.158	0.162	0.09
0.625	0.257 0.264	0.261	0.189
1.25	0.406 0.42	0.413	0.341
2.5	0.698 0.696	0.697	0.625
5	1.204 1.244	1.224	1.152
10	1.979 2.017	1.998	1.926
20	2.735 2.789	2.762	2.69

9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	20	1.0	0.05	4.8
2	20	4.8	0.06	1.3
3	20	17.6	0.43	2.4

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	24	1.4	0.04	2.6
2	24	5.2	0.13	2.5
3	24	18.4	0.36	1.9

9.3 Recovery

The recovery of human SERPINE1 spiked to three different levels throughout the range of the assay in human samples were evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:300	84	72-98
	1:600	85	74-99
Human serum	1:160	87	79-98
	1:320	85	77-98
Cell culture supernatant	1:30	91	84-98
	1:60	89	88-91
Cell lysate	1:16	87	80-95
	1:32	89	87-93

9.4 Sample values

Human serum and plasma samples from volunteers were evaluated for human SERPINE1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Rang (ng/mL)
Human plasma(n=15)	341.1	17.5-512.9
Human serum (n=16)	304.8	173.3-415.2

Cell culture supernatant:

HepG2 (human hepatocellular carcinoma cells) were cultured in DMEM supplemented with 10% fetal bovine serum, 2.5 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernatant was removed, assayed for human SERPINE1, and measured 38.6 ng/mL.

A549 were cultured in DMEM supplemented with 10% fetal bovine serum, 2.5 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernatant was removed, assayed for human SERPINE1, and measured 63.8 ng/mL.

HUVEC were cultured in DMEM supplemented with 10% fetal bovine serum, 2.5 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernatant was removed, assayed for human SERPINE1, and measured 465.4 ng/mL.

Cell lysate:

	A549 lysate	HUVEC lysate
SERPINE1 /Total protein (ng/mg)	4.7	11.7

9.5 Sensitivity

The minimum detectable dose of human SERPINE1 is 0.16 ng/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

9.6 Linearity

To assess the linearity of the assay, Samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The plasma and serum samples were initially diluted 1:10, cell culture supernatant was initially diluted 1:2）

		Human plasma (Sample Diluent PT 4)	Human serum (Sample Diluent PT 4)	Cell culture supernatant (Sample Diluent PT 1)	Cell lysate (Sample Diluent PT 1)
1:2	Average% of Expected	95	100	95	84
	Range (%)	93-98	95-103	94-96	82-85
1:4	Average% of Expected	104	100	98	99
	Range (%)	104-105	100-101	96-100	92-106
1:8	Average% of Expected	100	100	101	100
	Range (%)	99-101	100-101	100-103	100-101
1:16	Average% of Expected	98	99	105	106
	Range (%)	98-99	97-102	103--108	97-114

10. References

1. Huang J. et al.(2012). Blood. 20: 4873-81.

2. Kohler HP. et al. (2000). N Engl J Med. 342: 1792-801.

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4. Look MP. et al. (2002). J Natl Cancer Inst. 16;94(2):116-28.

5. Ulisse S. et al. (2009). Curr Cancer Drug Targets. 9(1):32-71.