

## Human SERPINE1 Sandwich ELISA Kit Datasheet

For the quantitative detection of human SERPINE1 concentrations in serum, plasma, cell culture supernatants and cell lysates.

### General Information

Catalogue Number	KE00109
Product Name	Human SERPINE1 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	0.312-20 ng/mL
Tested applications	Quantification ELISA

### Database Links

Entrez Gene	5054
SwissProt	P05121

### Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	<b>Unopened Kit:</b> Store at 2-8°C for 6 months or -20°C for 12 months.  <b>Opened Kit:</b> All reagents stored at 2-8°C for 7 days.  <b>Please use a new standard for each assay.</b>
Protein standard - 20 ng/bottle; lyophilized*	2 bottles	
Detection antibody, HRP-conjugated (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 4 - 30 mL/bottle. For serum and plasma	1 bottle	
Sample Diluent PT 1 - 30 mL/bottle. For cell culture supernatants and cell lysates	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Extraction Reagent - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

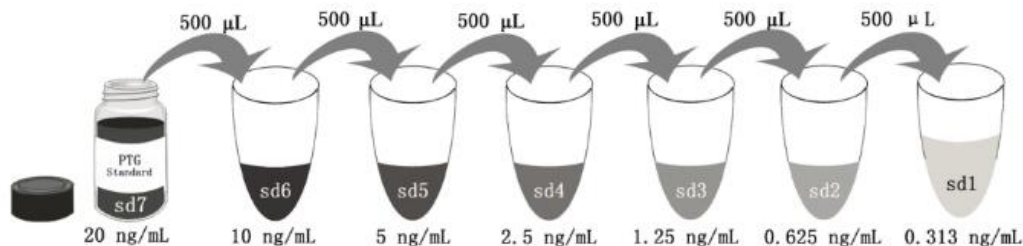
**NB: Do not use the kit after the expiration date.**

Sample Diluent PT 4 is for protein standard, serum and plasma samples.

Sample Diluent PT 1 is for protein standard, cell culture supernatants and cell lysates.

Detection Diluent is for Detection antibody .

\*Add 1 mL Sample Diluent PT 4 or PT 1 in protein standard. This reconstitution gives a stock solution of 20 ng/mL.



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	<b>1000 µL</b>	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## Product Description

KE00109 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The SERPINE1 ELISA kit is to be used to detect and quantify protein levels of endogenous SERPINE1. The assay recognizes human SERPINE1. An antibody specific for SERPINE1 has been pre-coated onto the microwells. The SERPINE1 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another horseradish peroxidase (HRP)-conjugated antibody specific for SERPINE1 is added to detect the captured SERPINE1 protein. For signal development, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

## 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/macrophages, 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.

## Sample Preparation

The serum or plasma, cell culture supernatants, cell lysates samples may require proper dilution to fall within the range of the assay. 1:80 or 1:160 dilution is recommended for serum or plasma, 1:16 or 1:32 dilution is recommended for cell culture supernatants, 1:8 or 1:16 dilution is recommended for cell lysates.

## Safety Notes

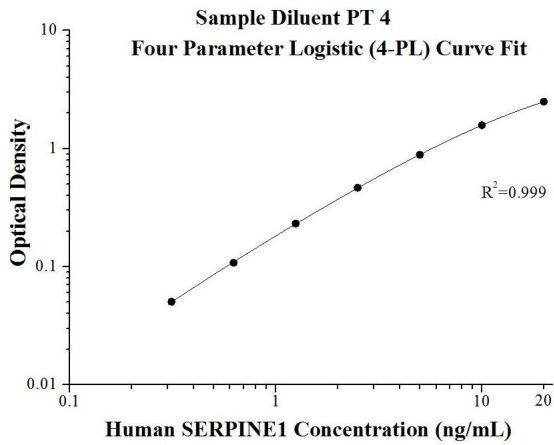
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## Assay Procedure Summary

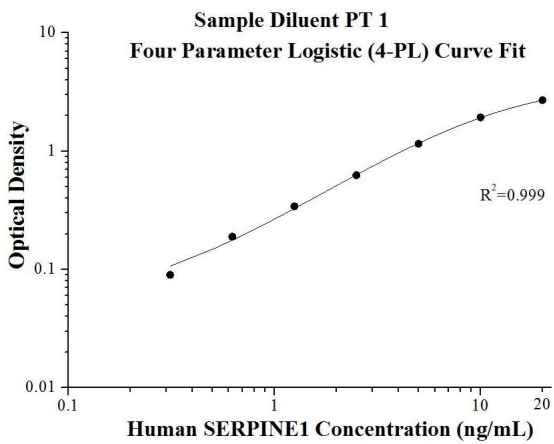
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Detection antibody, HRP-conjugated Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
4	Stop 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.				

## Example data

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



(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



(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

## 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

## Recovery

The recovery of SERPINE1 spiked to three different levels in four samples 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 supernatants	1:30	91	84-98
	1:60	89	88-91
Cell lysates	1:16	87	80-95
	1:32	89	87-93

## Sample Values

Serum and plasma samples from healthy volunteers were evaluated for 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 supernatants:

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  $\mu$ g/mL streptomycin sulfate. An aliquot of the cell culture supernatants 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  $\mu$ g/mL streptomycin sulfate. An aliquot of the cell culture supernatants 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  $\mu$ g/mL streptomycin sulfate. An aliquot of the cell culture supernatants was removed, assayed for human SERPINE1, and measured 465.4 ng/mL.

### cell lysates:

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

## 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.

## Linearity

To assess the linearity of the assay, Samples were diluted with the appropriate Sample Diluent PT 4 or PT 1 to produce samples with values within the dynamic range of the assay. (The plasma and serum samples were initially diluted 1:10, cell culture supernatants was initially diluted 1:2)

		Human plasma (PT 4)	Human serum (PT 4)	Cell culture supernatants (PT 1)	Cell lysates (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

## References

1. Huang J. et al.(2012). Blood. 20: 4873-81.
2. Kohler HP. et al. (2000). N Engl J Med. 342: 1792-801.
3. Festa A. et al. (2006). Circulation. 113: 1753-9.
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.