proteintech®
Antibodies | ELISA kits | Proteins

colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human SERPINA3 in serum and plasma.

general information

Catalogue Number	KE00037
Product Name	SERPINA3 ELISA Kit
Species cross-reactivity	Human SERPINA3
Range (calibration Range)	1 - 64 ng/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**12** (Human)
SwissProt	**P01011** (Human)

kit components & storage

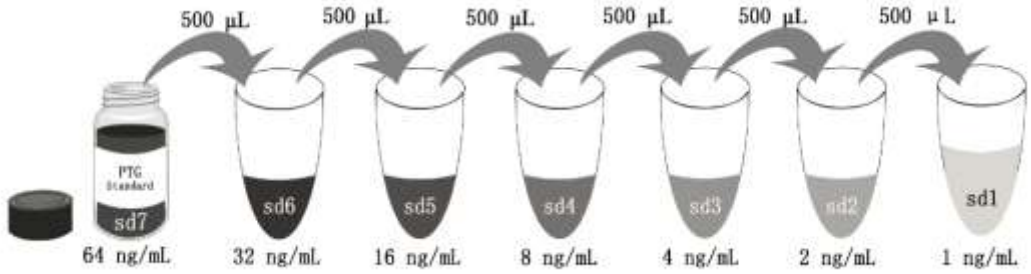
Microplate - antibody coated 96-well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Standard - 128 ng/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-a - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	3 pieces	

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

Sample Diluent PT 1-a is for standard and samples.

Detection Diluent is for Detection antibody and HRP-conjugated antibody.

*Add **2 mL** Sample Diluent PT 1-a in standard. This reconstitution gives a stock solution of 64 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 1-a	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00037 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The SERPINA3 ELISA kit is to be used to detect and quantify protein levels of endogenous SERPINA3. The assay recognizes human SERPINA3. An antibody specific for SERPINA3 has been pre-coated onto the microwells. The SERPINA3 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for SERPINA3 is added to detect the captured SERPINA3 protein. For signal development, horseradish peroxidase (HRP)-conjugated antibody is added, 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 450nm with the correction wavelength set at 630 nm.

background

Human SerpinA3, also known as Alpha 1-antichymotrypsin (AACT), is a member of the serine protease inhibitor class, and is a plasma alpha globulin glycoprotein which increases in the blood during the inflammatory process. SerpinA3, is also an inhibitor of neutrophil cathepsin G, mast cell chymases and pancreatic chymotrypsin. SerpinA3 is produced primarily in the liver, and is identified as an acute-phase inflammatory protein. SerpinA3 deficiency has been associated with liver disease. SerpinA3 has also been implicated in the pathology of a number of devastating human diseases including chronic obstructive pulmonary disease (COPD), Parkinson's disease (PD), Alzheimer's disease (AD), Stroke, Cystic Fibrosis, Cerebral Haemorrhage and Multiple System Atrophy.

sample preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

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.

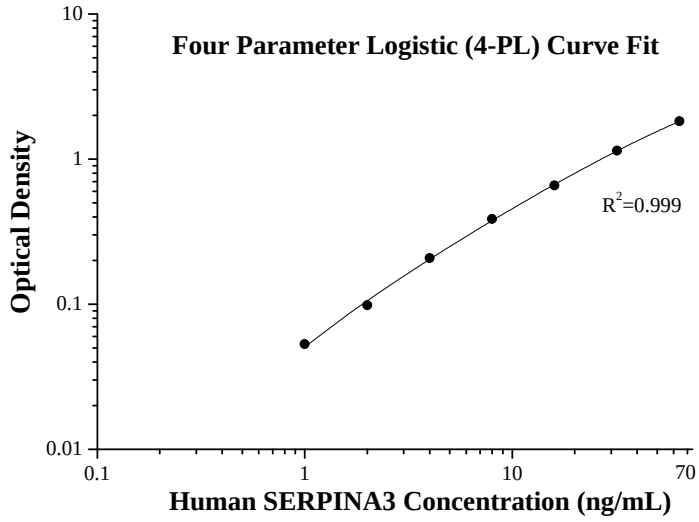
USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com

assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	60 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP 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
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

typical 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	0.088	0.0845	—
	0.081		
1	0.144	0.1375	0.053
	0.131		
2	0.196	0.183	0.0985
	0.17		
4	0.294	0.292	0.2075
	0.29		
8	0.481	0.4705	0.386
	0.46		
16	0.761	0.7415	0.657
	0.722		
32	1.245	1.228	1.1435
	1.211		
64	1.949	1.9055	1.821
	1.862		

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			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (ng/mL)	28.9	5.6	1.2	26.8	5.3	1.4
SD	1.6	0.4	0.1	1.9	0.3	0.1
CV%	5.5	6.9	9.7	6.9	6.1	8.6

USA: Proteintech@ptglab.com
UK & Europe: Europe@ptglab.com
China: Proteintech-CN@ptglab.com
ptglab.com

recovery

The recovery of SERPINA3 spiked to three different levels in four samples throughout the range of the assay in human plasma averaged 99%, ranging from 82%-113%.

sample values

Twenty-four serum and plasma samples from healthy volunteers were evaluated for human SERPINA3 in this assay. Twenty-three samples measured less than the lowest standard, 1 ng/mL. One sample measured 8 ng/mL. No medical histories were available for the donors used in this study.

sensitivity

The minimum detectable dose of human SERPINA3 is 0.55 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, three samples were spiked with high concentrations of SERPINA3 in human plasma and diluted with the appropriate **Sample Diluent PT 1-a** to produce samples with values within the dynamic range of the assay. (The samples were initially diluted 1:3)

		Human plasma
1:2	Average% of Expected	85
	Range (%)	81-90
1:4	Average% of Expected	92
	Range (%)	85-96
1:8	Average% of Expected	99
	Range (%)	89-105
1:16	Average% of Expected	105
	Range (%)	97-110

references

1. Law RHP. et al., 2006, Genome Biol.7: 216.
2. Kalsheker NA. Et al., 1996, Int J Biochem Cell Bio. 28: 961-964.
3. Janciauskiene S. Et al., 2001, Biochim Biophys Acta. 1535: 221-235.
4. Eriksson, S. et al., 1995, Proc. Natl. Acad. Sci. USA. 92: 2313-2317.
5. Ikari, Y. et al., 2001, J. Biol. Chem. 276: 11798-11803.