Human Alpha-1-Antitrypsin/SERPINA1 Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE00337

Size: 96T

Sensitivity: 0.04 ng/mL

Range: 0.39-25 ng/mL

Usage: For the quantitative detection of human Alpha-1-Antitrypsin/SERPINA1 concentrations in serum, plasma, urine, saliva and human milk.

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

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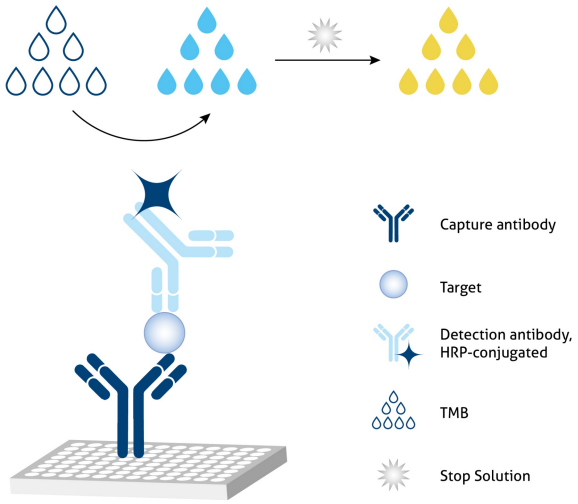
1. Background

Alpha-1-antitrypsin (AAT) is the gene for a protein called SERPINA1, which is a serine protease inhibitor whose targets include elastase, plasmin, thrombin, trypsin, chymotrypsin, and plasminogen activator. AAT is a glycoprotein synthesized primarily by hepatocytes, with smaller amountssynthesized by intestinal epithelial cells, neutrophils, pulmonary alveolar cells and macrophages. AAT is the most abundant, endogenous serine protease inhibitor in blood circulation and it has been implicated in regulating vital fluid phase biological events such as blood coagulation, fibrinolysis, complement activation, apoptosis, reproduction, tumor progression and inflammatory response. The primary function of AAT is thought to be the inactivation of neutrophil elastase and other endogenous serine proteases. Defects in SERPINA1 can cause emphysema or liver disease.

2. Principle



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.

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4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 50 ng/bottle; lyophilized	2 bottles	
Detection antibody, HRP-conjugated (100×) - 120 µL/vial*	1 vial	
Sample Diluent PT 4B1 - 30 mL/bottle	3 bottles	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 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°C. 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°C. Avoid repeated freeze-thaw cycles.

6.3 Urine: Collect urine samples and centrifuge for 20 minutes at 1000xg. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

6.4 Saliva: Collect saliva samples and centrifuge for 5 minutes at 10,000xg. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

6.5 Human Milk: Collect milk samples and Centrifuge for 15 minutes at 1000xg at 2-8°C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately.

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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.

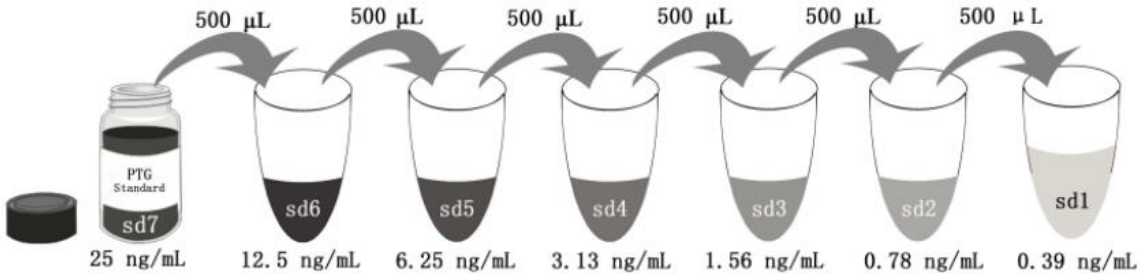
7.2 Detection Antibody, HRP-conjugated(1X): Dilute 100X Detection Antibody, HRP-conjugated 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody, HRP-conjugated + 990 µL Detection Diluent (Centrifuge the 100X Detection Antibody solution, HRP-conjugated 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: 1:1,280,000 or 1:2,560,000 is recommended for human serum and plasma; 1:128 or 1:256 is recommended for urine; 1:200 or 1:400 is recommended for saliva; 1:3,200 or 1:6,400 is recommended for human milk.

7.5 Standard Serial Dilution:

Add 2 mL Sample Diluent PT 4B1 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 4B1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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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°C 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 2 hours at 37°C.

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°C.

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	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
3	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.				

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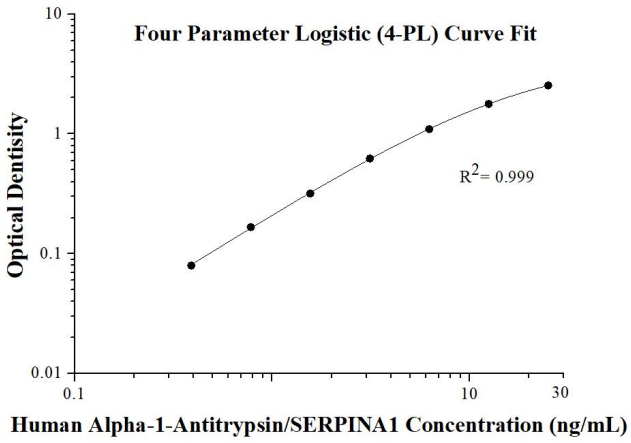
9. Validation Data

9.1 Standard curve

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



(ng/mL)	O.D	Average	Corrected
0	0.0389 0.0340	0.0365	-
0.39	0.1192 0.1136	0.1164	0.0799
0.78	0.2094 0.1978	0.2036	0.1671
1.56	0.3545 0.3551	0.3548	0.3183
3.13	0.6740 0.6465	0.6603	0.6238
6.25	1.1477 1.1186	1.1332	1.0967
12.5	1.8433 1.7922	1.8178	1.7813
25	2.6064 2.5354	2.5709	2.5344

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	10.53	0.37	3.56
2	20	2.48	0.06	2.28
3	20	0.64	0.02	3.29

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	24	11.15	0.52	4.64
2	24	2.68	0.11	4.21
3	24	0.67	0.04	6.02

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9.3 Recovery

The recovery of human Alpha-1-Antitrypsin/SERPINA1 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average % of Expected	Range (%)
Human plasma	1:2,560,000	93	77-112
	1:5,120,000	87	77-92
Urine	1:128	95	83-121
	1:256	94	85-123
Saliva	1:800	105	89-115
	1:1,600	86	83-91
Human milk	1:12,800	87	82-97
	1:25,600	85	74-97

9.4 Sample values

Human plasma - Human plasma samples were evaluated for the presence of human Alpha-1-Antitrypsin/SERPINA1 in this assay.

Sample Type	Mean (**µg/mL**)	Range (**µg/mL**)
Human plasma (n=16)	8,580.07	4,743.91-14,892.02

Urine/Saliva/Human milk - Urine, saliva and human milk samples were evaluated for the presence of human Alpha-1-Antitrypsin/SERPINA1 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Urine (n=7)	525.14	45.15-1,125.13
Saliva (n=8)	1,244.83	366.11-3,377.23
Human milk (n=6)	41,184.49	7,066.20-70,693.59

9.5 Sensitivity

The minimum detectable dose of human Alpha-1-Antitrypsin/SERPINA1 is 0.04 ng/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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9.6 Linearity

To assess the linearity of the assay, human plasma, urine, saliva and human milk were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(Human plasma samples were initially diluted 1:320,000. Urine samples were initially diluted 1:16. Saliva samples were initially diluted 1:100. Human milk samples were initially diluted 1:1,600.)

		Human plasma	Urine	Saliva	Human milk
1:2	Average% of Expected	100	100	100	100
	Range (%)	-	-	-	-
1:4	Average% of Expected	101	104	101	96
	Range (%)	99-104	103-105	98-105	93-101
1:8	Average% of Expected	96	107	101	94
	Range (%)	90-101	101-112	95-109	93-96
1:16	Average% of Expected	100	105	104	94
	Range (%)	90-116	102-109	97-107	90-97

9.7 Specificity

This assay recognizes natural and recombinant human Alpha-1-Antitrypsin/SERPINA1.

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:

Serpin C1

Serpin A3

Serpin A5

Serpin A4

Serpin F2

Serpin A9

10. References

1. Dasí F. (2024). Med Clin (Barc). 162(7):336-342.

2. Lechowicz U. et al. (2020). Int J Mol Sci. 21(23):9187.

3. Torres-Durán M. et al. (2018). Orphanet J Rare Dis. 13(1):114.

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