abcam

Product datasheet

Human alpha 1 Antitrypsin ELISA Kit (SERPINA1) ab189579

SimpleStep ELISA

7 References 6 Images

Overview

Product name Human alpha 1 Antitrypsin ELISA Kit (SERPINA1)

Detection methodColorimetric

Precision Intra-assay

Sample	n	Mean	SD	CV%
Overall	5			3.9%

Inter-assay

Sample	n	Mean	SD	CV%	
Overall	3			2.1%	

Sample type Serum, Hep Plasma, EDTA Plasma, Cit plasma

Assay type Sandwich (quantitative)

Sensitivity 13 ng/ml

Range 31.25 ng/ml - 2000 ng/ml

Recovery Sample specific recovery

Sample type	Average %	Range
Serum	96	92% - 101%
Hep Plasma	99	94% - 102%
EDTA Plasma	77	70% - 87%
Cit plasma	85	83% - 86%

Assay time 1h 30m

Assay duration One step assay

1

Species reactivity

Product overview

Reacts with: Human

Does not react with: Goat, Cow, Pig, Cynomolgus monkey, Macaque monkey

Human alpha 1 Antitrypsin ELISA Kit (SERPINA1) (ab189579) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of alpha 1 Antitrypsin (SERPINA1) protein in cit plasma, hep plasma, serum, and edta plasma. It uses our proprietary SimpleStep ELISA® technology. Quantitate Human alpha 1 Antitrypsin (SERPINA1) with 13 ng/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (<u>ab203359</u>) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

ASSAY SPECIFICITY

This kit recognizes both native and recombinant human alpha 1 Antitrypsin protein in serum, plasma, and cell culture supernatant, cell and tissue extract samples only.

Normal human urine and saliva samples tested negative for this kit.

CROSS REACTIVITY

Human alpha 1 Anti-chymotrypsin protein and human PAI protein were prepared at 2,000 ng/mL and 1,000 ng/mL respectively and assayed for cross reactivity. No cross-reactivity was observed.

INTERFERENCE

Human alpha 1 Anti-chymotrypsin protein and human PAI protein were prepared at 2,000 ng/mL and 1,000 ng/mL respectively and assayed for and tested for interference. No interference with was observed.

SPECIES REACTIVITY

This kit recognizes human alpha 1 Antitrypsin protein.

Other species reactivity was determined by measuring alpha 1 Antitrypsin (1:10,000) serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species: Mouse, Rat, Hamster, Guinea Pig, Rabbit, Dog, Goat, Pig and Cow.

CALIBRATION

This immunoassay is calibrated against a highly purified human alpha 1 Antitrypsin. The NIBSC/WHO unclassified purified human alpha 1 Antitrypsin preparation 05/162 was evaluated in this kit.

The dose response curve of the unclassified standard alpha 1 Antitrypsin parallels the SimpleStep standard curve. To convert sample values obtained with the SimpleStep Human alpha 1 Antitrypsin kit to approximate NIBSC 05/162 units, use the equation below.

NIBSC (05/162) approximate value (ng/mL) = 0.3649 x SimpleStep Human alpha 1 Antitrypsin value (ng/mL).

alpha 1 Antitrypsin (SERPINA1) is a serine protease inhibitor found in a variety of biological fluids. While it can inhibit trypsin, chymotrypsin, and plasminogen activator, it's main target is elastase. Alpha 1 antitrypsin plays a critical role in the lung by inhibiting the activity of elastase, produced by neutrophils during inflammatory responses. The aberrant form plays a role in coagulation and insulin induced NO synthesis. Alpha 1 antitrypsin deficiency results in emphysema and may also result in liver disease.

Platform Microplate

Properties

Notes

Storage instructions

Store at +4°C. Please refer to protocols.

Components	1 x 96 tests	10 x 96 tests
10X Human alpha 1 Antitrypsin Capture Antibody	1 x 600µl	10 x 600µl
10X Human alpha 1 Antitrypsin Detector Antibody	1 x 600µl	10 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml	1 x 200ml
Antibody Diluent 4BI	1 x 6ml	10 x 6ml
Human alpha 1 Antitrypsin Lyophilized Purified Protein	2 vials	20 vials
Plate Seals	1 unit	10 units
Sample Diluent NS (ab193972)	1 x 50ml	2 x 250ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit	10 units
Stop Solution	1 x 12ml	1 x 120ml
TMB Development Solution	1 x 12ml	1 x 120ml

plasmin and thrombin. Irreversibly inhibits trypsin, chymotrypsin and plasminogen activator. The aberrant form inhibits insulin-induced NO synthesis in platelets, decreases coagulation time and has proteolytic activity against insulin and plasmin.

Short peptide from AAT: reversible chymotrypsin inhibitor. It also inhibits elastase, but not trypsin. Its major physiological function is the protection of the lower respiratory tract against proteolytic destruction by human leukocyte elastase (HLE).

Tissue specificity Ubiquitous. Expressed in leukocytes and plasma.

Involvement in disease Alpha-1-antitrypsin deficiency
Sequence similarities Belongs to the serpin family.

DomainThe reactive center loop (RCL) extends out from the body of the protein and directs binding to the target protease. The protease cleaves the serpin at the reactive site within the RCL, establishing

a covalent linkage between the carboxyl group of the serpin reactive site and the serine hydroxyl

of the protease. The resulting inactive serpin-protease complex is highly stable.

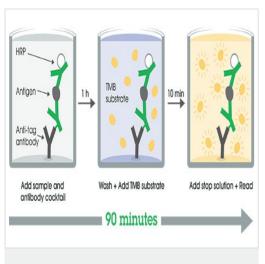
Post-translational
N-glycosylated. Differential glycosylation produces a number of isoforms. N-linked glycan at Asnmodifications
107 is alternatively di-antennary, tri-antennary or tetra-antennary. The glycan at Asn-70 is diantennary with trace amounts of tri-antennary. Glycan at Asn-271 is exclusively di-antennary.
Structure of glycans at Asn-70 and Asn-271 is Hex5HexNAc4. The structure of the antennae is
Neu5Ac(alpha1-6)Gal(beta1-4)GlcNAc attached to the core structure Man(alpha1-6)[Man(alpha13)]Man(beta1-4)GlcNAc(beta1-4)GlcNAc. Some antennae are fucosylated, which forms a Lewis-

X determinant.

Proteolytic processing may yield the truncated form that ranges from Asp-30 to Lys-418.

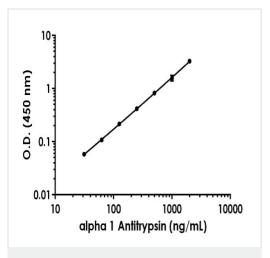
Cellular localizationSecreted. Endoplasmic reticulum. The S and Z allele are not secreted effectively and accumulate intracellularly in the endoplasmic reticulum and Secreted, extracellular space, extracellular matrix.

Images



Other - Human alpha 1 Antitrypsin ELISA Kit (SERPINA1) (ab189579)

SimpleStep ELISA technology allows the formation of the antibodyantigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



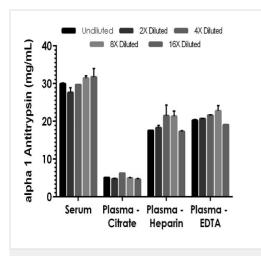
Background-subtracted data values (mean +/- SD) are graphed.

100 100 5000	230023800	14307	7.353V
Concentration (ng/mL)	O.D 4	Mean	
	1	2	O.D
0	0.076	0.078	0.077
31.25	0.135	0.135	0.135
62.5	0.185	0.185	0.185
125	0.280	0.305	0.293
250	0.481	0.512	0.496
500	0.896	0.911	0.903
1,000	1.515	1.791	1.653
2,000	3.439	3.278	3.359

Example of alpha 1 Antitrypsin standard curve.

Example of human alpha 1 Antitrypsin standard curve in Sample Diluent NS. The alpha 1 Antitrypsin standard curve was prepared as described. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

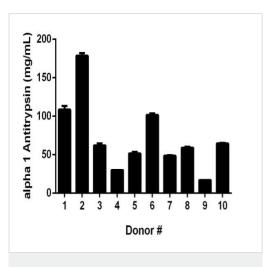
Standard curve



Interpolated concentrations of native alpha 1

Antitrypsin in human serum and plasma samples.

The concentrations of alpha 1 Antitrypsin were measured in duplicates, interpolated from the alpha 1 Antitrypsin standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 0.005%, plasma (citrate) 0.025%, plasma (heparin) 0.005%, and plasma (EDTA) 0.005%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean alpha 1 Antitrypsin concentration was determined to be 30 mg/mL in serum, 5 mg/mL in plasma (citrate), 19 mg/mL in plasma (heparin) and 21 mg/mL in in plasma (EDTA).



Observed alpha 1 Antitrypsin levels in individual donor normal Human serum (n=10).

The mean alpha 1 Antitrypsin concentration was determined to be 72 mg/mL with a range of 17-181 mg/mL.

Dilution Factor	Interpolated value	0.005 % Human Serum	0.025 % Human Plasma (Citrate)	0.005% Human Plasma (EDTA)	0.005% Human Plasma (Heparin)
11	ng/mL	1,497	1,271	878	1,012
Undiluted	% Expected value	100	100	100	100
^	ng/mL	689	595	457	517
2	% Expected value	92	94	104	102
	ng/mL	371	388	268	270
4	% Expected value	99	122	122	107
8	ng/mL	196	155	133	142
	% Expected value	105	98	121	112
16	ng/mL	99	72	54	60
	% Expected value	106	91	98	94

Linearity of dilution.

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native alpha 1 Antitrypsin was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

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