



Human alpha-1 Antitrypsin ELISA Kit

Vertrieb:

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Hinweis/Note:

Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

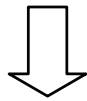
Thank you for choosing Assaypro.

Assay Summary

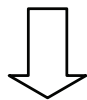
Add 25 μ l of Standard/ Sample
and 25 μ l of Biotinylated Protein per well.
Incubate 2 hours.



Wash, then add
50 μ l of SP Conjugate per well.
Incubate 30 minutes.



Wash, then add
50 μ l of Chromogen Substrate per well.
Incubate 20 minutes.



Add 50 μ l of Stop Solution per well.
Read at 450 nm immediately.

AssayMax Human alpha-1 Antitrypsin ELISA Kit

Catalog No. EA5001-1
Sample Insert/Reference Only

Introduction

Alpha-1 antitrypsin (A1AT) is a protein that protects the lungs. The liver usually makes the protein and releases it into the bloodstream. A1AT is a major protease inhibitor that controls tissue degradation. A reduction of A1AT levels can cause a change in collagen metabolism (1). A1AT inhibits neutrophil elastase release into the lungs during inflammatory states (2). A1AT deficiency is an uncommon genetic disease (3) that can lead to emphysema (4), hepatitis, cirrhosis (5), and chronic obstructive pulmonary disease (COPD) (6).

Principle of the Assay

The AssayMax Human alpha-1 Antitrypsin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human A1AT in plasma and serum samples. This assay employs a quantitative competitive enzyme immunoassay technique that measures human A1AT in less than 3 hours. A polyclonal antibody specific for human A1AT has been pre-coated onto a 96-well microplate with removable strips. A1AT in standards and samples is competed with a biotinylated A1AT sandwiched by the immobilized antibody and streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated protein, and SP conjugate) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

Reagents

- **Human alpha-1 Antitrypsin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human A1AT.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human alpha-1 Antitrypsin Standard:** Human A1AT in a buffered protein base (80 µg, lyophilized).
- **Biotinylated Human alpha-1 Antitrypsin:** 1 vial, lyophilized.
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard and Biotinylated Protein at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:800 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes, and remove serum. Dilute samples 1:800 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 80 µg of Human alpha-1 Antitrypsin Standard with 2 ml of MIX Diluent to generate a 40 µg/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (40 µg/ml) 1:4 with MIX Diluent to produce 10, 2.5, 0.625, 0.156, and 0.039 µg/ml solutions. MIX Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[A1AT] (µg/ml)
P1	Standard (40 µg/ml)	40.00
P2	1 part P1 + 3 parts MIX Diluent	10.00
P3	1 part P2 + 3 parts MIX Diluent	2.500
P4	1 part P3 + 3 parts MIX Diluent	0.625
P5	1 part P4 + 3 parts MIX Diluent	0.156
P6	1 part P5 + 3 parts MIX Diluent	0.039
P7	MIX Diluent	0.000

- **Biotinylated Human alpha-1 Antitrypsin (2x):** Reconstitute Biotinylated Human alpha-1 Antitrypsin with 4 ml MIX Diluent to produce a 2-fold stock solution. Allow the biotin to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution should be further diluted 1:2

with MIX Diluent. Any remaining solution should be frozen at -20°C and used within 30 days.

- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25 µl of Human alpha-1 Antitrypsin Standard or sample per well, and immediately add 25 µl of Biotinylated Human alpha-1 Antitrypsin to each well (on top of the standard or sample) and mix gently. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 20 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

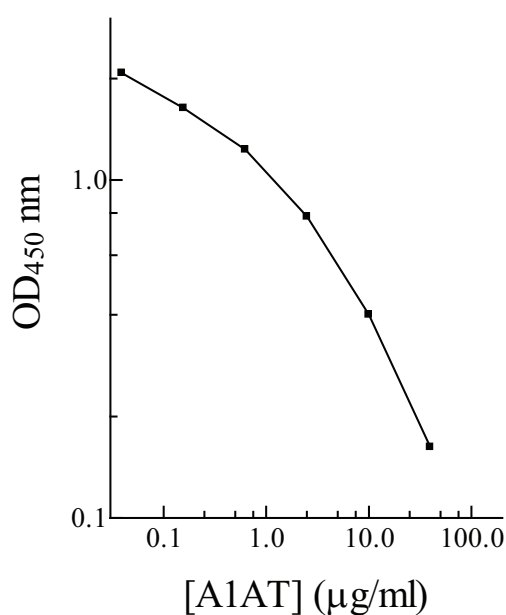
Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

H. A1AT Standard Curve



Precision, Sensitivity and Specificity

- The minimum detectable dose of A1AT is typically ~ 0.03 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.5% and 7.2% respectively.
- **Kit standard has been calibrated against WHO International Standard.**

Cross-Reactivity

Species	% Cross Reactivity
Monkey	< 5%
Mouse	None
Rat	None
Swine	None
Beagle	None
Bovine	None
Rabbit	None
Human	100%

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:400	104%	105%
1:800	100%	99%
1:1600	96%	97%

Recovery

Standard Added Value	0.1 – 10 µg/ml
Recovery %	87 – 110%
Average Recovery %	98%

Reference Value

- Normal human alpha-1 antitrypsin plasma levels range from 1.0 to 3.6 mg/ml.

References

- (1) Hauck EW *et al.* (2004) *Eur Urol.* 46(5): 623-8; discussion 628.
- (2) Chappell *et al.* (2004) *Hum Mutat.* 24(6): 535-6.
- (3) Strange C *et al.* (2006) *Respiration* 73(2):185-90
- (4) Abboud RT *et al.* (2005) *Treat Respir Med.* 4(1): 1-8.
- (5) Kok, KF *et al.* (2005) *Ned Tijdschr Geneeskd.* 149(37): 2057-61.
- (6) Teramoto S (2007) *Intern Med.* 46(2): 77-9.

Version 2.4

Related Products

- EA5101-1 AssayMax Human alpha-1 Antitrypsin ELISA Kit (Urine, Cell Culture, Saliva, CSF, and Milk samples)