Human α1-Antitrypsin ELISA Kit

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Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

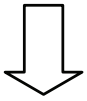
The datasheet is only a first information.
The relevant datasheet is included with the product.

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

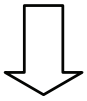
Thank you for choosing Assaypro.

Assay Summary

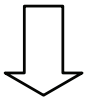
Add 50 µl of Standard/Sample per well.
Incubate 2 hours.



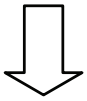
Wash, then add 50 µl of
Biotinylated Antibody per well.
Incubate 1 hour.



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



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



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

Assay Template

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

AssayMax Human α1-Antitrypsin ELISA Kit

Catalog No. EA5101-1
Sample Insert/Reference Only

Introduction

Alpha-1-Antitrypsin (α1AT) is a protein that protects the lungs. The liver usually makes the protein and releases it into the bloodstream. Human α1AT is a major protease inhibitor that controls tissue degradation. A reduction of α1AT levels can cause a change in collagen metabolism (1). Human α1AT inhibits neutrophil elastase release into the lungs during inflammatory states (2). Human α1AT 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 α1-Antitrypsin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of α1AT in human urine, milk, saliva, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures α1AT in less than 4 hours. A polyclonal antibody specific for α1AT has been pre-coated onto a 96-well microplate with removable strips. Human α1AT in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for α1AT, which is recognized by a 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 antibody, 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 and the biotinylated antibody 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 α1AT Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human α1AT.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human α1AT Standard:** Human α1AT in a buffered protein base (300 ng, lyophilized).
- **Biotinylated Human α1AT Antibody (50x):** A 50-fold biotinylated polyclonal antibody against human α1AT (140 µl).
- **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, 2 bottles).
- **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 and Biotinylated Antibody 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 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

- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute urine samples 1:20 into MIX Diluent or within the range of 1:2 to 1:200, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:2000 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.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:400 into MIX Diluent or within the range of 1:40 to 1:4000, 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 300 ng of Human α1AT Standard with 3 ml of MIX Diluent to generate a standard solution of 100 ng/ml. 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 (100 ng/ml) 1:4 with MIX Diluent to produce 25, 6.25, 1.563, and 0.391 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[α1AT] (ng/ml)
P1	Standard (100 ng/ml)	100.0
P2	1 part P1 + 3 parts MIX Diluent	25.00
P3	1 part P2 + 3 parts MIX Diluent	6.250
P4	1 part P3 + 3 parts MIX Diluent	1.563
P5	1 part P4 + 3 parts MIX Diluent	0.391
P6	MIX Diluent	0.000

- **Biotinylated Human α1AT Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **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, working standards 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 50 µl of Human α1AT Standard or sample per well. 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 Biotinylated Human α1AT Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per 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 the 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

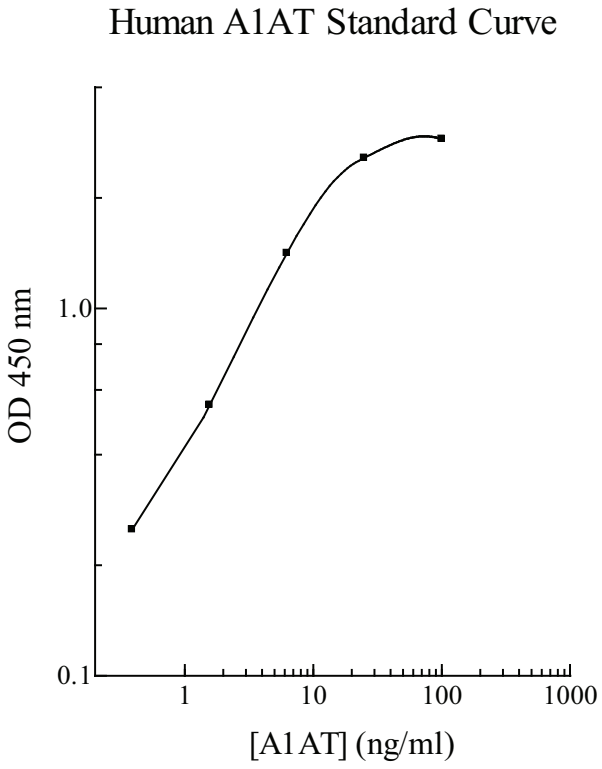
unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

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 four-parameter or log-log 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.



Sensitivity and Specificity

- The minimum detectable dose of α1AT is typically ~ 0.3 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.0% respectively.
- **Kit standard has been calibrated against WHO International Standard**.

Linearity

	Average Percentage of Expected Value
Sample Dilution	**Urine**
1:10	91%
1:20	97%
1:40	106%

	Average Percentage of Expected Value
Sample Dilution	**Milk**
1:1000	90%
1:2000	97%
1:4000	104%

	Average Percentage of Expected Value
Sample Dilution	**Saliva**
1:200	87%
1:400	98%
1:800	103%

Recovery

Standard Added Value	1.56 – 25 ng/ml
Recovery %	84 – 111%
Average Recovery %	98.5%

Cross-Reactivity

Species	**% Cross Reactivity**
Bovine	None
Canine	None
Mouse	None
Monkey	<5%
Rat	None
Swine	None
Human	100%

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) *Respriration* 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 2.5

Related Products

- EA5001-1 AssayMax Human α1-Antitripsin ELISA Kit (Plasma and Serum Samples)