Human AT III
ELISA Kit

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

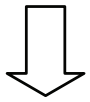
The datasheet is only a first information.
The relevant datasheet is included with the product.

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

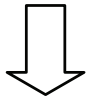
Thank you for choosing Assaypro.

Assay Summary

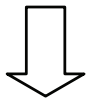
Add 50 µl of Standard/ Sample per well.
Incubate 2 hours.



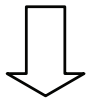
Wash, then add 50 µl of
Biotinylated Antibody per well.
Incubate 1 hour.



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



Wash, then add 50 µl of
Chromogen Substrate per well.
Incubate 12 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 Antithrombin III ELISA Kit

Catalog No. EA3301-1
Sample Insert/Reference Only

Introduction

The serine protease inhibitor antithrombin III (AT III), the most important natural inhibitor of thrombin activity, has been shown to exert marked anti-inflammatory properties and proven to be efficacious in experimental models of sepsis, septic shock, and disseminated intravascular coagulation (1). It has often been recommended for the therapy of septic patients as it provides anticoagulant and anti-inflammatory actions (2). AT III deficiency is a rare hereditary disease that predisposes to thrombo-embolic complications (3). AT III levels are positively correlated with plasma total cholesterol levels, plasma low-density lipoprotein cholesterol levels, plasma triglycerides and D-dimer levels (4).

Principle of the Assay

The AssayMax Human Antithrombin III ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human AT III in urine, milk, saliva, and cell culture supernatant samples. This assay employs a quantitative sandwich enzyme immunoassay technique, which measures AT III in less than 4 hours. A monoclonal antibody specific for AT III has been pre-coated onto a 96-well microplate with removable strips. AT III in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for AT III, 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 AT III Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human AT III.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **Human AT III Standard:** Human AT III in a buffered protein base (400 ng, lyophilized).
- **Biotinylated Human AT III Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against AT III (140 µl).
- **Streptavidin-Peroxidase Conjugate (SP conjugate):** A 100-fold concentrate (80 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).
- **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 samples at -20°C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:2 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 1:10 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.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:200 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.
- **Human ATIII Standard:** Reconstitute the 400 ng of Human AT III Standard with 2 ml of MIX Diluent to generate a 200 ng/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 (200 ng/ml) 1:2 with equal volume of MIX Diluent to produce 100, 50, 25, 12.5, 6.25, and 3.125 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 the next 30 days.

Standard Point	Dilution	AT III (ng/ml)	AT III (mU/ml)
P1	Standard (200 ng/ml)	200.0	0.300
P2	1 part P1 + 1 part MIX Diluent	100.0	0.150
P3	1 part P2 + 1 part MIX Diluent	50.00	0.075
P4	1 part P3 + 1 part MIX Diluent	25.00	0.038
P5	1 part P4 + 1 part MIX Diluent	12.50	0.019
P6	1 part P5 + 1 part MIX Diluent	6.250	0.009
P7	1 part P6 + 1 part MIX Diluent	3.125	0.005
P8	MIX Diluent	0.000	0.000

- **Biotinylated Human AT III 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.
- **Streptavidin-Peroxidase 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 AT III Standard or sample per well, and cover wells 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 AT III 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.

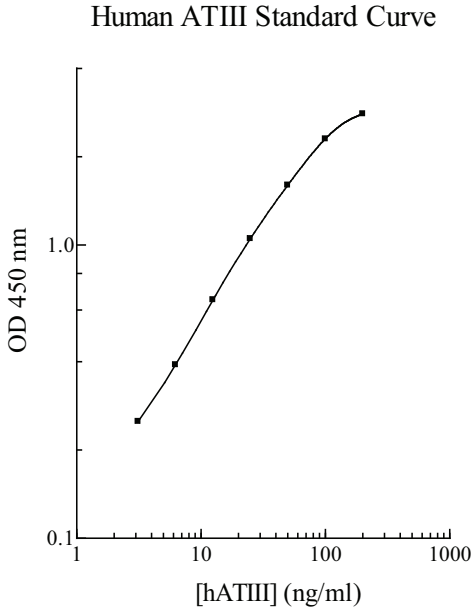
- Add 50 µl of Chromogen Substrate per well and incubate for about 12 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- 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.

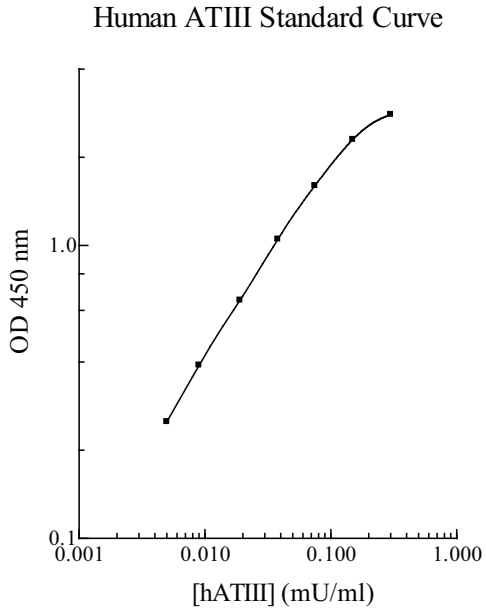
Data Analysis

- 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 4-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.





Performance Characteristics

- The minimum detectable dose of AT III is typically ~ 3 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.8 % and 7.1% respectively.
- **Kit standard has been calibrated against WHO International Standard.**

Linearity

	Average Percentage of Expected Value
Sample Dilution	**Urine**
No dilution	90%
1:2	100%
1:4	102%

	Average Percentage of Expected Value
Sample Dilution	**Milk**
1:100	92%
1:200	98%
1:400	104%

	Average Percentage of Expected Value
Sample Dilution	**Saliva**
1:5	91%
1:10	98%
1:20	102%

Recovery

Standard Added Value	6.25 – 50 ng/ml
Recovery %	92 – 112%
Average Recovery %	98.5%

Cross-Reactivity

Species	**% Cross Reactivity**
Canine	None
Monkey	90%
Mouse	None
Rat	None
Swine	None
Rabbit	None
Bovine	None

References

(1) Oelschläger C *et al.* (2002) *Blood* 99(11):4015-20.
(2) Kulka PJ *et al*. (2001) *Anasthesiol Intensivmed Notfallmed Schmerzther*. 36(3): 143-53.
(3) Takahashi J. *et.al.* (2003) *Ann Thorac Cardiovasc Surg.* 9(3):192-6.
(4) Erem C *et al.* (2005) *Med Princ Pract.* 14(1): 22-30

Version 5.8

Related Products

- EA3303-1 AssayMax Human AT III ELISA Kit (Plasma and Serum samples)
- EMA3301-1 AssayMax Mouse AT III ELISA Kit (Plasma, Serum, and Cell Culture samples)