Human TAT Complex ELISA Kit

Vertrieb:
L O X O GmbH Immunbiologie Biochemie, Produkte und Systeme
Postfach 11 30 69215 Dossenheim
Telefon +49 (0) 62 21 - 86 80 23 **FAX** +49 (0) 62 21 - 86 80 255
E-Mail: info@loxo.de **Internet:** www.loxo.de

Assaypro LLC
30 Triad South Drive
St. Charles, MO 63304
T (636) 447-9175
F (636) 447-9475
www.assaypro.com

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.

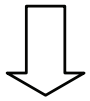
Thank you for choosing Assaypro.

Symbol Key

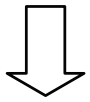
Consult instructions for use.

Assay Summary

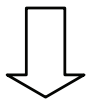
Add 50 µl of Standard/ Sample per well.
Incubate 2 hours.



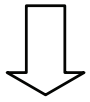
Wash, then add
50 µl of Biotinylated Antibody per well.
Incubate 1 hour.



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 Thrombin-Antithrombin (TAT) Complex ELISA Kit

Catalog No. ET1020-1
Sample Insert/Reference Only

Introduction

Thrombin-antithrombin (TAT) complex formed following the neutralization of thrombin by antithrombin III (ATIII) have been used as a surrogate marker for thrombin generation (1). High plasma levels of TAT complexes have been suggested to alter hemostatic activation in argentine hemorrhagic fever (2), chronic dialysis patients (3), and toxemia of pregnancy (4). Whereas, low plasma levels of TAT complexes are found in type 1 (insulin-dependent) diabetes (5), neonatal respiratory distress syndrome (6), and primary untreated cancer (7). TAT complexes are a useful marker to predict morphological changes in chronic aortic dissection (8).

Principle of the Assay

The AssayMax Human TAT Complex ELISA (Enyzme-Linked Immunosorbent Assay) kit is designed for detection of human TAT complexes in plasma, milk, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures TAT complexes in less than 4 hours. A monoclonal antibody specific for antithrombin has been pre-coated onto a 96-well microplate with removable strips. TAT complex in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for thrombin, 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, standard, 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 Antithrombin Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human antithrombin.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human TAT Complex Standard:** Human TAT complex in a buffered protein base (180 ng, lyophilized).
- **Biotinylated Human Thrombin Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against thrombin (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 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 and assay. The undiluted samples can be stored at -20°C or below for up to 30 days. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Cell Culture Supernatants**: Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. The undiluted samples can be stored at -20°C or below for up to 30 days. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. The undiluted samples can be stored at -20°C or below for up to 30 days. 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 TAT Complex Standard:** Reconstitute the 180 ng of Human TAT Complex Standard with 1.5 ml of MIX Diluent to generate a 120 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 1:3 with MIX Diluent to produce 40, 13.33, 4.444, and 1.481 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	[TAT] (ng/ml)
P1	Standard (120 ng/ml)	120.0
P2	1 part P1 + 2 parts MIX Diluent	40.00
P3	1 part P2 + 2 parts MIX Diluent	13.33
P4	1 part P3 + 2 parts MIX Diluent	4.444
P5	1 part P4 + 2 parts MIX Diluent	1.481
P6	MIX Diluent	0.000

- **Biotinylated Human Thrombin 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, 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 50 µl of Human TAT Complex 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 Thrombin 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 20 minutes or till the optimal 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

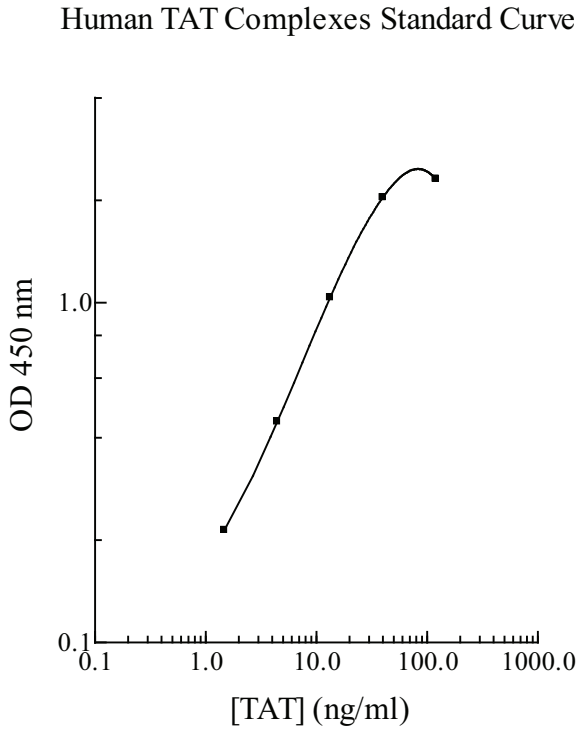
after stopping the reaction for about 10 minutes, which will reduce the readings.

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.



Precision, Sensitivity and Specificity

- The minimum detectable dose of TAT complex is typically ~ 1.4 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.1% respectively.

Linearity

	Average Percentage of Expected Value
Sample Dilution	**Plasma**
No dilution	98%
1:2	102%
1:4	106%

	Average Percentage of Expected Value
Sample Dilution	**Milk**
No dilution	98%
1:2	95%
1:4	96%

Standard Added Value

Standard Added Value	4.0 – 40 ng/ml

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Bovine	None
Monkey	<40%
Mouse	None
Rat	<15%
Swine	<15%
Rabbit	None
Human	100%

Reference Value

- The normal human plasma level of TAT Complex is 0.5 – 10 ng/ml.

References

(1) Diquelou A *et al.* (1994) *Blood* 84(7): 2206-13
(2) Heller MV *et al.* (1995) *Thromb Haemost.* 73(3): 368-73
(3) Kario K *et al.* (1992) *Thromb Res.* 67(1): 105-13
(4) Terao T *et al.* (1991) *Gynecol Obstet Invest.* 31(2): 74-85
(5) Ibbotson SH *et al.* (1995) *Thromb Haemost.* 73(2): 243-6
(6) Schmidt B *et al*. (1992) *Am Rev Respir Dis.* 145(4 Pt 1): 767-70
(7) Nanninga PB *et al*. (1990) *Thromb Haemost.* 64(3): 361-4
(8) Iyano K *et al.* (2004) *Ann Thorac Cardiovasc Surg* 10(2): 106-112

Version 8.5

Related Product

- EMT1020-1 AssayMax Mouse Thrombin-Antithrombin Complex ELISA Kit (Plasma and Cell Culture samples)