AssayMax Human Thrombin-antithrombin (TAT) Complexes ELISA Kit

Catalog # ET1020-1

Introduction

Thrombin-antithrombin (TAT) complexes formed following the neutralization of thrombin by antithrombin III (AT) have been used as a surrogate marker for thrombin generation (1). High plasma level of TAT complexes has been suggested to alter hemostatic activation in argentine hemorrhagic fever (2), chronic dialysis patients (3), and toxemia of pregnancy (4). Whereas low plasma level of TAT complexes is 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).

Principal of the Assay

The AssayMax TAT complexes ELISA kit is designed for detection of human TAT complexes in plasma, serum and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures TAT complexes in 4 hours. A polyclonal antibody specific for Thrombin has been pre-coated onto a microplate. TAT complexes in standards and samples are sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for Anti-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

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **Thrombin Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against Thrombin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.

- **TAT Complexes Standard:** Human TAT complexes in a TAT depleted plasma base buffer (360 ng, lyophilized).
- **Biotinylated Antithrombin Antibody (70x):** A 70-fold biotinylated polyclonal antibody against Antithrombin (110 µl).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 200-fold concentrate (60 µl).
- **Sample Diluent Concentrate (2x)**: A 2-fold concentrated TAT depleted plasma base buffer (30 ml).
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (20 ml).
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2 - 8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2 - 8^{0}C. Store reconstituted standard at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 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 2,000x g for 10 minutes and assay. Dilute samples 1:2 into Sample Diluent and assay. Samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000x g for 10 minutes. Dilute samples 1:2 into Sample Diluent and assay. Samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2,000x g for 10 minutes to remove debris. Collect supernatants and assay. Dilute samples 1:2 into Sample Diluent and assay. Samples can be stored at -20^{0}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. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

- **TAT Complexes Standard:** Reconstitute the 360 ng of human TAT Complexes Standard with 1 ml of Sample Diluent to generate a stock solution of 360 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the Standard solution (360 ng/ml) 1:3 with Sample Diluent to produce 120, 40, 13.33, 4.44, 1.48 and 0.49 ng/ml. Sample Diluent serves as the zero standard (0 ng/ml).

Standard Point	Dilution	[TAT] (ng/ml)
P1	1 part Standard (360 ng/ml)	360.00
P2	1 part P1 + 2 part Sample Diluent	120.00
P3	1 part P2 + 2 part Sample Diluent	40.00
P4	1 part P3 + 2 part Sample Diluent	13.33
P5	1 part P4 + 2 part Sample Diluent	4.44
P6	1 part P5 + 2 part Sample Diluent	1.48
P7	1 part P6 + 2 part Sample Diluent	0.49
P8	Sample Diluent	0.000

- **Biotinylated Antithrombin Antibody (70x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:70 with EIA Diluent.
- **Sample Diluent Concentrate (2x)**: Dilute the Sample Diluent 1:2 with reagent grade water
- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent 1:10 with reagent grade water.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer Concentrate 1:10 with reagent grade water.
- **SP Conjugate (200x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:200 with EIA Diluent.

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 (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells and incubate for 2 hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Biotinylated TAT Antibody to each well and incubate for 60 minutes.
- Wash five times with 200 µl of Wash Buffer as 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 five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 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.

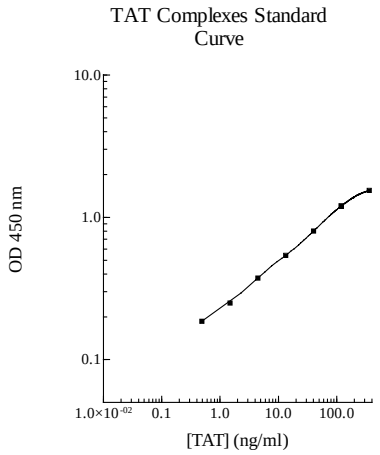
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the 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 curve fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the sample 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 TAT complexes is typically less than 400 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 6.3 % and 8.2% respectively.
- No significant cross-reactivity or interference was observed.

References

(1) Diquelou A *et al.* (1994) *Blood* 84(7): 2206-13
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(3) Kario K *et al.* (1992) *Thromb Res.* 67(1): 105-13
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(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

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