AssayMax Human Thrombin-antithrombin (TAT) Complexes ELISA Kit

Catalog No. 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, milk, and cell culture supernatants. 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 microplate. TAT complexes in standards and samples are 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

- 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

- **Antithrombin Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against Antithrombin.
- **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 buffered protein base (360 ng lyophilized).
- **Biotinylated Thrombin Antibody (25x):** A 25-fold concentrated biotinylated polyclonal antibody against thrombin (280 µl).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).

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- **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

- Store kit at 2 - 8^{0}C or -20^{0}C upon arrival up to the expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8^{0}C. Store reconstituted reagents 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, 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 2000 x g for 10 minutes and assay.
  The undiluted samples can be stored at -20^{0}C or below for up to 1 month. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)
- **Cell Culture Supernatants**: Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay.
  Samples can be stored at -20^{0}C or below for up to 1 month. 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^{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.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8^{0}C.
- **TAT Complexes Standard:** Reconstitute the 360ng of human TAT Complexes Standard with 1 ml of MIX Diluent to generate a standard solution of 360 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 1:3 with MIX Diluent to produce 120, 40, 13.33, 4.44 and 1.48 ng/ml. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20^{0}C.

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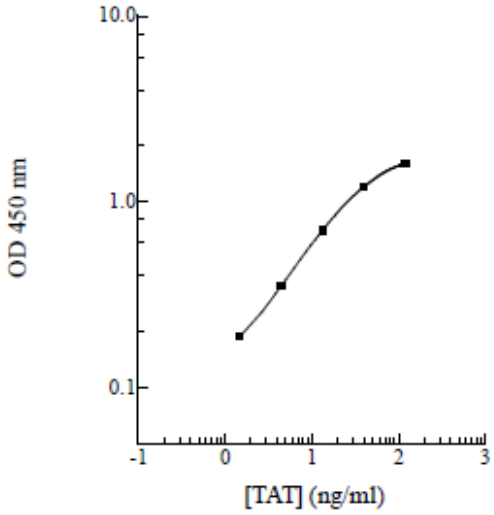
Data Analysis

- 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.

Human TAT Complexes Standard Curve



Precision, Sensitivity and Specificity

- The minimum detectable dose of TAT complexes is typically less than 1 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9 % and 7.2 % respectively.

Linearity

	Average Percentage of Expected Value
Sample Dilution	**Plasma**
No dilution	102%
1:2	104%
1:4	101%

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	Average Percentage of Expected Value
Sample Dilution	**Milk**
No dilution	97%
1:2	95%
1:4	94%

Recovery

Standard Added Value	2 - 20 ng/ml
Recovery %	83-115 %
Average Recovery %	99 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	< 1
Bovine	None
Monkey	< 5 (suggest dilution 1:2 for plasma)
Mouse	None
Rat	None
Swine	< 1

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

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