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# IMUBIND® TAT ELISA

**Product no. ADG833**

**Storage: 2 – 8°C** **For Research Use Only!**

***PRODUCT INSERT*** ***ENGLISH***

## INTENDED USE

The IMUBIND® TAT ELISA is an enzyme-linked immunosorbent assay for the quantitative determination of thrombin-antithrombin III (TAT) complexes.

## EXPLANATION OF THE TEST

The conversion of prothrombin to thrombin is a key event in thrombus (clot) formation. Thrombin is a serine protease that acts on a wide variety of substrates in the coagulation pathway. Antithrombin III, an alpha 2 glycoprotein with a molecular weight of 58,000 kDa, is the major inhibitor of thrombin. Inactivation of thrombin by antithrombin III occurs by the formation of a covalent bond resulting in an inactive complex. This thrombin-antithrombin III complex (TAT) can be quantified in peripheral blood as an indicator of thrombin activation. Elevated plasma levels of TAT have been associated with a wide variety of active thrombotic events including disseminated intravascular coagulation (DIC), sepsis, multiple trauma, pregnancy complications (pre-eclampsia), deep vein thrombosis (DVT), and malignancies. TAT levels have also been found to be elevated in many patients with disturbances in global coagulation when routine coagulation parameters are in the sub-normal or normal range.

## PRINCIPLE OF THE METHOD

Diluted plasma samples are added to microwells coated with a monoclonal antibody against thrombin. During an incubation period, TAT complexes present in the sample will bind to the antibody coated to the wells. Following a washing step, a biotinylated monoclonal anti-antithrombin III antibody is added to the microwells and binds to the TAT complexes captured on the plate during a short incubation period. A streptavidin-horseradish peroxidase conjugate (SA-HRP) is added to the microwells to complete the formation of the antibody-enzyme detection complex. Following another washing step, the addition of a perborate-3,3'-5,5'-tetramethylbenzidine (TMB) substrate and its subsequent reaction with the HRP present generates a blue colored solution. The reaction is stopped by adding citrate stop solution, which turns the solution color yellow. Measuring the solution absorbance at 450 nm and extrapolating the value with those of a standard curve determines the level of TAT in the diluted plasma sample.

## REAGENTS

| | |
|---|---|
| **MTP** | **Antibody Coated Microtiter plate,** MTP-96 (12x8) well |
| **WASH** | **Wash buffer**, 50 ml, 1 vial (20x concentrate) |
| **SBUF** | **Sample buffer**, 15 ml, 1 vial (ready-to-use) |
| **CBUF** | **Conjugate buffer**, 50 ml, 1 vial (ready-to-use) |
| **STD1-5** | **TAT Standard plasmas 1-5**, conc. see label, 1 ml, 1 vial each (lyophilized) |
| **POS** | **Positive control plasma**, conc. see label, 1 ml, 1 vial (lyophilized) |
| **AB** | **Detection Antibody**, biotinylated mAb, 120 µl, 1 vial (100x concentrate) |
| **CON** | **Enzyme conjugate**, SA-HRP, 120 µL (100x concentrate) |
| **TMB** | **Substrate**, 12 ml, 1 vial (ready-to-use) |
| **STOP** | **Stop solution**, 6 ml, 1 vial (ready-to-use) |

## PRECAUTIONS

Source material for some of the reagents in this kit is of human origin. This material has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA approved methods. As no known test method provides complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, reagents should be handled as recommended for any potentially infectious human specimen. Discard all waste associated with test specimens and human source reagents in a biohazard waste container.

Not for internal use in humans or animals. Do not use the kit components beyond the expiration date. Do not mix reagents from different kit lots. Avoid microbial contamination of the reagents. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Do not pipette reagents by mouth or ingest reagents. Wear laboratory coat and disposable gloves throughout the test procedure and wash hands thoroughly afterwards. Handle gently; avoid splashing, foam, or aerosol formation.

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|---|---|---|---|
| WASH<br>CBUF<br>AB<br>CON | Warning |  | H317, P280, P333+P313 |
| STOP | Warning |  | H314, P303+P361+P353, P305+P351+P338, P310 |

**Hazard Statements**:

H314 Causes severe skin burns and eye damage

H317 May cause an allergic skin reaction.

**Precautionary Statements**:

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection

P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention.

P303 + P361 + P353 IF ON SKIN (or hair): Remove/ Take off immediately all contaminated clothing. Rinse skin with water/shower.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 Immediately call a POISON CENTRE or doctor/physician.

## REAGENT PREPARATION AND STORAGE

Unopened and lyophilized reagents are stable until the expiration date printed on the box when stored as instructed.

**MTP** **Antibody coated microwells:** Once removed from the foil pouch, the microwell strips must be used within 30 minutes. Unused strips may be stored at 2°-8°C for 4 weeks when sealed in the original pouch with the desiccant present, protected from any moisture.

**WASH** **Wash buffer:** If crystals are visible, incubate the vial in a 37°C water bath a few minutes until the crystals are dissolved. Transfer the content to a 1 liter bottle and fill up the concentrate to 1 liter with filtered deionized/distilled water. Diluted Wash Buffer may be used for up to 4 weeks when stored at 2°-8°C.

**SBUF** **Sample buffer:** Supplied ready to use. Opened dilution buffer is stable for 3 month when stored at 2°-8°C.

**CBUF** **Conjugate buffer:** Supplied ready to use. Opened dilution buffer is stable for 3 month when stored at 2°-8°C.

**STD** **Standards:** Reconstitute the standard plasmas with 1 ml purified, deionised or distilled water, swirl the contents gently and allow the vials to stand at room temperature for at least 15 minutes to ensure complete dissolution. The lyophilised plasma is stable until the date indicated on the vial label when stored at 2° - 8°C. Once reconstituted, the plasma will remain stable for 3 month when stored at -20 °C. See vial label for lot-specific concentration.

**POS** **Positive control plasma:** Reconstitute the control plasma with 1 ml purified, deionised or distilled water, swirl the content gently and allow the vial to stand at room temperature for at least 15 minutes to ensure complete dissolution. The lyophilised plasma is stable until the date indicated on the vial label when stored at 2° - 8°C. Once reconstituted, the plasma will remain stable for 3 month when stored at -20 °C.

**AB** **Detection antibody:** Supplied as a concentrate, dilute the Detection Antibody 1:100 with Conjugate Buffer just prior to use. For using all 96 microwells at one time, dilute 100 µL of Detection Antibody to 10 mL in Conjugate Buffer. If not all 96 microwells are used, dilute 10 µL of Detection Antibody to 1 mL in Conjugate Buffer for each 8-microwell strip that will be used. Working strength Detection Antibody is stable for 4 hours at 2°-8°C. Discard any unused working strength Detection Antibody. Opened antibody is stable for 3 month when stored in the dark at 2°-8°C.

**CON** **Enzyme conjugate:** Supplied as a concentrate, dilute the Enzyme Conjugate 1:100 with Conjugate Buffer just prior to use. For running all 96 microwells at one time, dilute 100 µL of Enzyme Conjugate to 10 mL in Conjugate Buffer. If not all 96 microwells are used, dilute 10 µL of Enzyme Conjugate to 1 mL in Conjugate Buffer for each 8-micro-well strip that will be used. Working strength Enzyme Conjugate is stable for 2 hours at 2°-8°C. Discard any unused working strength Enzyme Conjugate.

**TMB** **Substrate, TMB:** Supplied ready to use. Opened substrate is stable for 3 month when stored in the dark at 2° - 8°C.

**STOP** **Stop solution:** Supplied ready to use. Opened stop solution is stable for 3 month when stored at 2° - 8°C.

## SPECIMEN COLLECTION AND PREPARATION

EDTA or citrate collected platelet poor plasma may be used for this assay. See "Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation Assays; Approved Guidelines-Fourth Edition", NCCLS Document H21-A4, Vol. 23, No. 35, December 2003.
Citrate Plasma collection should be performed as follows:

1. Collect 9 parts of blood into 1 part of 3.2% (0.109 M) trisodium citrate anticoagulant solution.
2. Centrifuge the blood sample at 5,000 x g for 15 minutes.
3. Plasma should be stored at 2°-8°C and assayed within 4 hours.
4. Alternatively, aliquot plasma and store at –20°C. Do **not** subject to freeze-thaw cycles.
5. Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2°-8°C and assayed within 4 hours.

## PROCEDURE

### Materials Provided – See Reagents

### Material Required But Not Provided

0.22 µm filtered deionized $H_2O$
50-300 µL eight channel multi-pipette
0-200 µL, 200-1000 µL single pipettes
microwell plate reader for reading absorbance at 450 nm
microwell plate washer (optional), microwell plate shaker (optional)

### Preparing the TAT standards and control

**1.** Reconstitute the TAT standard and positive control plasmas as instructed under REAGENT PREPARATION.

Running standard, control and samples in duplicate is recommended.

### Preparing the Sample Dilutions

**2.** Dilute each plasma sample 1:20 with sample buffer (e.g. for duplicates: 12 µl plasma + 228 µl sample buffer).

### Assay Procedure

**3.** Open the foil pouch and remove the microwell strips/frame assembly. Remove the strips that will not be used, return them to the foil pouch and tightly reseal the pouch with the desiccant inside. Store the foil pouch at 2 - 8°C.

**4.** Pipette 100 µL of the standard, control or samples into separate microwells, cover with the acetate sheet and incubate for 1 hour at room temperature (18-25°C) on an orbital microwell plate shaker with agitation (at 250 rpm).

**5.** Empty the contents of the microwells and wash 4 times with Wash Buffer. Washing may be performed either using microwell plate washing equipment or manually (fill the wells with Wash Buffer with a pipette or squeeze bottle, wait three minutes, empty and remove droplets by tapping the plate 4-5 times face down against absorbing material).

**6.** Add 100 µL working strength Detection Antibody to each microwell, cover with the acetate sheet and incubate the wells at room temperature (18-25°C) for 1 hour on an orbital microwell plate shaker with agitation (at 250 rpm).

**7.** Wash the wells by repeating Step 5.

**8.** Add 100 µL of working strength Enzyme Conjugate to each microwell, cover with the acetate sheet and incubate for 1 hour at room temperature (18-25°C) on an orbital microwell plate shaker with agitation (at 250 rpm).

**9.** Wash the wells by repeating Step 5.

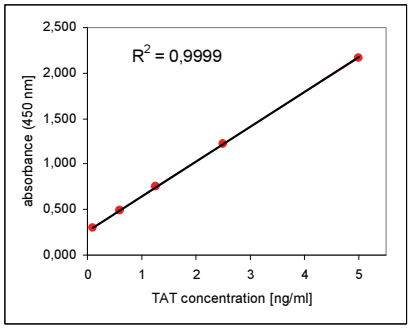
**10.** Add 100 µL of Substrate to each microwell immediately after the wash step, cover the wells with the acetate sheet and incubate for 10-15 minutes at room temperature (18°-25°C). A blue color will develop.

**11.** Stop the enzymatic reaction by adding 50 µL Stop solution to each microwell. Add the acid with the same speed and order as you added the substrate. Tap the sides of the microwell frame to ensure even distribution of the solution. The solution color will turn yellow. Read the absorbance on a microwell plate reader at a wavelength of 450 nm within 10 minutes.

## RESULTS

Construct a standard curve by plotting the mean absorbance value for each standard versus its corresponding concentration. A standard curve should be generated each time the assay is performed. The following standard curve is for demonstration purposes only.

### Representative Standard Curve




## CALCULATIONS

Determine the amount of TAT in the diluted plasma sample by interpolating directly from the standard curve. As the plasma sample was diluted 1:20 during its preparation, multiply the results by 20 in order to obtain the concentration of TAT in the neat plasma sample. Since the TAT positive control plasma was pre-diluted 1:20, multiply the results by 20 too. The calculation is:

$$[TAT]_{Plasma\ Sample} = [TAT]_{Diluted\ Test\ Sample} \times 20$$

Samples which yield absorbances above the highest standard must be retested in other dilutions. Sample dilutions (e.g 1:40) must be prepared using a normal plasma with a TAT content of < 4 ng/ml. The TAT content of the normal plasma must be taken into account in the calculation of the TAT concentration of the sample.

## QUALITY CONTROL

Patient samples should be tested along with the provided Positive control plasma. The measurement values for the samples can only be used if the value for the control is within the confidence interval.

## LIMITATIONS OF THE PROCEDURE

Platelet contamination in plasma samples may interfere with the assay results. Plasma samples must be free of platelets in order to have a valid result. Exercise great care in minimizing disruption of the platelet pellet while recovering the platelet poor plasma. Samples should not be frozen and thawed more than two times.

## EXPECTED VALUES

Each laboratory should establish its own normal range using the local population.
In a study using the IMUBIND TAT ELISA the TAT concentration of EDTA plasma from normal adult donors (n=36) was: mean < 1.2 ng/ml, reference range: (5th -95th percentile) < 1.2 to 3.3 ng/ml.

## PERFORMANCE CHARACTERISTICS

### Precision

The intra- and inter-assay coefficients of variations (CV) for this ELISA have been estimated to be 3.3 % and 12.9 % respectively.

### Specificity

The assay is specific for human thrombin/antithrombin III complex.

## BIBLIOGRAPHY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