Version 28-04/25

User's Manual

Human Thrombin-Antithrombin complex Matched Antibody Pair

REF **ABPR-L027**

Σ **5 x 96 tests**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This antibody pair set comes with matched antibody pair to detect and quantify protein level of Human Thrombin-Antithrombin complex

Principles of Testing

Affinity-purified antibody to human thrombin is coated onto the wells of a microtitre plate. Any remaining binding sites on the plastic wells are blocked with bovine serum albumin. The plates are washed and plasma or other fluids are applied. The coated antibody will capture the thrombin and thrombin-inhibitor complexes in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to ATIII is added to the plate to bind to the captured TAT complexes. After washing the plate to remove unbound conjugated antibody, the peroxidase activity is expressed by incubation with o-phenylenediamine (OPD). After a fixed development time the reaction is quenched with the addition of H_2SO_4 and the colour produced is quantified using a microplate reader. The colour generated is proportional to the concentration of TAT complex present in the sample.

Reagents And Materials Provided

1. Capture Antibody (yellow): 0.5 ml of polyclonal affinity purified anti-thrombin antibody for coating plates.
2. Detecting Antibody (red): 0.5 ml of peroxidase conjugated affinity-purified polyclonal anti-ATIII antibody for detection of captured TAT complex.
3. Conjugate Diluent (neutral): Five neutral-capped tubes each containing 10 ml of universal diluent for peroxidase conjugated antibody. Each vial sufficient for one 96-well plate.

Note: Reagents are sufficient for at least 5×96 well plates using recommended protocols. Antibodies are supplied in a 50% (v/v) glycerol solution for storage at 2 -8°C. Keep vials tightly capped.

Materials Required But Not Supplied

1. Coating Buffer: 50 mM Carbonate
2. PBS:(base for wash buffer and blocking buffer)
3. Wash Buffer: PBS-Tween(0.1%, v/v)
4. Blocking Buffer: PBS-BSA(2%, w/v)
5. Sample Diluent: HBS-BSA-T20
6. Substrate Buffer: Citrate-Phosphate buffer pH 5.0
7. OPD Substrate:(o-Phenylenediamine. 2HCl) TOXIC!
8. Stopping Solution: 2.5 M H_2SO_4
9. Materials for making TAT reference standards: - Human thrombin and antithrombin III; - Antithrombin(ATIII) deficient plasma.
10. Other: Microplates; Microplate washer; Microplate reader.

Storage

-10 to -20°C

Assay Procedure

1. **Preparation of TAT complex reference standard:** Purified ATIII(295 µg/mL = 5 µM) in 20 mM Tris-HCl, 0.15 M NaCl, pH 7.4, 1 mM EDTA and 0.05 U/mL heparin, is incubated with a limiting amount of thrombin(37 µg/mL = 1 µM) at 37°C for 10 minutes. Complete inhibition should be confirmed by plasma clot time or chromogenic assay. If all thrombin is completely inhibited, the concentration of TAT complex is 1 µM. A series of standards can be made by diluting this stock TAT complex into TAT-poor plasma(plasma shown to be low in TAT by independent assay) or ATIII-deficient plasma. Prepare standards containing 600, 200, 60 and 20 pM respectively.
2. **Coating of plates:** Dilute the capture antibody 1/100 in coating buffer(preferably in a polypropylene tube) and immediately add 100 µl to every well in the plate. Incubate 2 hours at room temperature or overnight at 2-8°C.
3. **Blocking:** Empty contents of plate and add 150 µl of blocking buffer to every well and incubate for 90 minutes at 22°C. Wash plate 3 times with wash buffer.
4. Standards and Test Samples: Test samples and the reference standards prepared above are each diluted 1/4 in sample diluent. Apply 100 µl/well and incubate plate at 22°C for 120 minutes. Wash plate 3 times with wash buffer.
5. **Detecting Antibody:** Dilute the detecting antibody 1/100 in conjugate diluent provided and apply 100 µl to each well. Incubate plate at 22°C for 60 minutes. Wash plate 3 times with wash buffer.
6. **OPD Substrate:** Apply 100 µl of freshly prepared OPD substrate to every well. Allow colour to develop for 10-15 minutes then stop colour reaction with the addition of 50 µl/well of 2.5 M H2SO4. The plate can be read at wavelength of 490 nm.