Human Antithrombin Matched Pair Antibodies for EIA

(5 x 96 Tests)

REF ATIII-EIA

For Research Use Only
Not for Use in Diagnostic Procedures
For in vitro use only

Store at 2 to 8˚ C

INTENDED USE:

Human Antithrombin (AT) Matched Pair Antibodies for EIA are intended for use with in-house enzyme-linked immunosorbent assays for measuring human AT in plasma, or in any biological fluid where human AT can be present. The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

SUMMARY:

Antithrombin is a 58.2 kDa serine protease inhibitor produced by the liver and composed of a single chain glycoprotein. AT inhibits thrombin, FIXa, FXa, and FXIa; it circulates in the plasma at a concentration of about 200 µg/mL (approx.. 3.5 µM).

ASSAY PRINCIPLE:

The diluted plasma sample or biological fluid is introduced into one of the microwells of a micro-ELISA plate which has been pre-coated with anti-human AT antibody. When present in the added material, AT binds to the anti-human polyclonal antibody. Following a washing step, the remaining bound antibodies are revealed with a anti-human peroxidase conjugated detection antibody, which reacts specifically with human AT. Following another washing step, the peroxidase substrate, o-Phenylenediamine (OPD) in presence of hydrogen peroxide (H_2O_2), is introduced and a yellow color develops. The color turns orange when the reaction is stopped with sulfuric acid. The color developed is directly proportional to the amount of AT present in the tested sample.

REAGENTS:

Required Materials provided (enough for 5x96 Tests):

- **C: Capture Antibody (ATIII-EIA-C)**. 1 vial of 0.5 mL affinity purified polyclonal antibody specific for human AT. For coating plates. Yellow cap.
- **D: Detecting Antibody (ATIII-EIA-D)**. 5 vials of 10 mL polyclonal antibody specific for human AT, coupled to peroxidase. For detecting captured AT. Neutral cap.

Required Materials not provided:

Optimum performance can be obtained when the following solutions and assay conditions are used.

- **Micro ELISA plates** with hydrophilic surface designed for high binding of IgG. For example, 96-well Immulon 4-HBX.
- **Coating Solution** (50mM Carbonate). Dissolve 1.59 g of Na_2Cl_3 and 2.93 g of $NaHCO_3$ in distilled water to a final volume of 1 L and adjust pH to 9.6. Store at 2-8°C for 1 month.
- **Phosphate-Buffered Saline** (PBS) [For preparation of sample diluent, wash and blocking solutions.] Dissolve 8.0 g NaCl, 1.15 g Na_2HPO_4, 0.2 g KH_2PO_4 and 0.2 g KCl in distilled water to a final volume of 1 L and adjust pH to 7.4. Store up to 1 month at 2-8°C, discard if there is evidence of microbial growth.
- **Wash Solution and Sample Diluent:** (PBS/Tween-20 0.1% v/v). Add 1.0 mL of Tween-20 to 1 L of PBS and adjust pH to 7.4. Store at 2-8°C up to 1 week.
- **Substrate Solution: (**Citrate-Phosphate buffer). Dissolve 2.6 g Citric Acid and 6.9 g Na_2HPO_4 in 450 mL distilled H_2O and adjust pH to 5.0 with Phosphoric acid or NaOH; add distilled water to final volume of 500 mL. Store at 2-8°C up to 1 month.
- **OPD Substrate**(o-phenylenediamine.2HCl) ☒ Toxic! 5 mg tablets: e.g. Sigma #P-6912. Prepare immediately before use. Dissolve 5 mg OPD in 12 mL Substrate Solution and then add 12 µL 30% H_2O_2. Do not store.
- **Stop Solution:** (2.5M H_2SO_4) ☒ Corrosive! Generates heat on dilution! Handle with great care. Avoid any skin and eye contact. Wear protective glasses and gloves when handling. Carefully add 13.9 mL 18M H_2SO_4 to 86 mL distilled H_2O. Store at room temperature for up to 1 month.
- **Reference standards** for Antithrombin which have the same matrix and anticoagulant as the samples to be tested
- Micro ELISA plate washing equipment and shaker.
- Plate reader with a wavelength set up at 490 nm.

PROCEDURE:

1. Coat ELISA plate: Dilute the Capture Antibody with Coating Solution *1/100* (use polypropylene tube) and immediately add 100 µL to every well in the plate. Incubate *2 hours at 22°C or preferable overnight at 2-8°C*.

2. Blocking: Blocking is not required under the conditions described. Wash plate 3X with Wash Solution.

3. Samples: Dilute AT Reference standard with Wash Solution/Sample Diluent *1/2000 (100%)* then serially dilute by halves down to *1/64000 (3.13%)*. Dilute sample plasmas or biological fluid with Sample Diluent *1/4000, 1/8000 and 1/16000*. Apply *100 µL* per well and incubate plate at *22°C for 90 minutes*. Wash plate 3X with Wash Solution/Sample Diluent. (Plasma samples should not be applied at dilutions lower than *1/100*, as falsely high readings may result.)

4. Detecting Antibody: Apply *100 µL* to each well. Incubate plate at *22°C for 60 minutes*. Wash plate 3X with Wash Solution.

5. OPD Substrate: Apply *100 µL* of freshly prepared OPD substrate to each well. Allow color to develop for *10-15 minutes* then stop color reaction with the addition of *50 µL* per well of Stop Solution. Read the plate at a wavelength of *490 nm*. [Optimal color development time is the time required to obtain A490 ≥ 1.000 for the 100% reference point, not to exceed *20 minutes*.]

Additional Notes:

- Do not shake plate during incubation.
- Do not allow the wells to become dry. Keep plate covered or in a humid chamber during incubations.
- Rheumatoid factor in samples may bind to the capture and/or detecting antibodies and cause interference in the ELISA assay.

6. Calibration Curve: On bi-logarithmic graph paper, plot the known AT concentrations on abscissa and the corresponding absorbance (A490) on ordinates in order to establish the calibration curve.

RESULTS:

From the constructed calibration curve, directly determine the Antithrombin concentration and multiply by the appropriate dilution factor.

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