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COA CHROM DIAGNOSTICA

Human tPA
Matched Pair Antibodies for EIA
(5 x 96 Tests)

Ref#: TPA-EIA

Store at -10 to -20°C

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

INTENDED USE:

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

SUMMARY:

Human tPA is a 68 kDa serine protease synthesized primarily in endothelial cells. tPA is beside uPA one of two major activators of Plasminogen. Activation of Plasminogen by tPA is dependent on the presence of Fibrin cofactor and occurs by cleavage after residue Arg560 to produce the two-chain active serine protease Plasmin. The activity of tPA is regulated by a very short half-life in circulation and by circulating PAI-1 and α2-Macroglobulin. Most of the tPA (<90%) is complexed with its primary inhibitor PAI, he normal tPA concentration in human plasma is reported from 20 ng/mL to 5 µg/mL depending on the assay used.

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 tPA antibody. When present in the added material, tPA binds to the anti-human polyclonal antibody. Following a washing step, the remaining bound antibodies are revealed with a detection antibody, anti-human peroxidase conjugate, which reacts specifically with human tPA. Following another washing step, the peroxidase substrate, o-Phenylenediamine (OPD) in presence of hydrogen peroxide (H_2O_2), is introduced and a yellow colour develops. The colour turns orange when the reaction is stopped with sulfuric acid. The colour developed is directly proportional to the amount of tPA present in the tested sample.

REAGENTS:

Required Materials provided (enough for 5x96 Tests):

C: Capture Antibody (TPA-EIA-C). 1 vial of 0.5 mL polyclonal affinity purified antibody specific for human tPA. For coating plates. Supplied in a 50% v/v glycerol solution. Yellow cap.

D: Detecting Antibody (TPA-EIA-D). 1 vial of 0.5 mL polyclonal antibody specific for human tPA, coupled to peroxidase. For detecting captured tPA. Supplied in a 50% v/v glycerol solution. Red cap.

Note: Antibodies are provided in a glycerol solution (50% v/v) and should be **stored at -10 to -20°C**. Vials should be tightly capped. Do not store in frost-free freezers

Antibodies can be centrifuged briefly in a micro-centrifuge to gather residual reagent from the cap and walls of the tube.

In their original packaging, before use, when stored at -10 to -20°C, the unopened antibodies are stable until the expiration date printed on the vial.

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_2CO_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 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:** (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.
- **Blocking Solution:** (PBS/BSA 1% w/v). Dissolve 2.5 g of Bovine Serum Albumin in 200 mL of PBS and adjust pH to 7.4; add PBS to final volume of 250 mL. Aliquot and store frozen at -20°C.
- **Sample Diluent:** (HEPES/BSA/Tween-20). Dissolve 5.95 g HEPES (free acid), 1.46 g NaCl, and 2.5 g Bovine Serum Albumin (Sigma, RIA grade) in 200 mL distilled H_2O; add 0.25 mL of Tween-20 and adjust pH to 7.2 with NaOH; add distilled water to final volume of 250 mL. Aliquot and store frozen at -20°C for up to 6 months.
- **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 tPA 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 overnight at 2-8°C*.

2. Blocking: Empty contents of plate and add *150 µL* of Blocking Solution to every well and incubate for *60 minutes at* 22°C. This

step blocks any remaining binding sites on the plastic wells. Wash plate 3X with Wash Solution.

3. Samples: Reconstitute tPA Standard and tPA deficiency plasma according to manufacturers instructions. Dilute tPA standard in tPA deficiency plasma to achieve standard plasmas with final tPA concentrations of 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/mL.
Dilute standard plasmas and sample plasmas with Sample Diluent 1/4 and 1/8. Apply 100 µL per well in duplicate and incubate plate @ 22°C for 90 minutes. Wash plate 3X with Wash Solution. (Plasma samples should not be applied at dilutions lower than *1/2*, as falsely high readings may result.)

4. Detecting Antibody: Dilute the Detecting Antibody with Sample Diluent *1/100* and apply *100 µL* to each well. Incubate plate at *22°C for 90 minutes*. Wash plate 3X with Wash Solution.

5. OPD Substrate: Apply *100 µL* of freshly prepared OPD substrate to each well. Allow colour to develop for *5-10 minutes* then stop colour reaction with the addition of *50 µL* per well of Stop Solution. Read the plate at a wavelength of *490 nm*. (Optimal colour 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 tPA 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 tPA concentration and multiply by the appropriate dilution factor.

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