Human tPA Antigen
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) Antibodies for EIA are intended for use with in-house enzyme-linked immunosorbent assays for measuring human 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 68KDa 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 Arg90 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 hydrophilic surface designed for high binding of IgG. For example, 96-well Immulon 4-HBX.

ANTIBODIES:
Antibodies are stable until the expiration date printed on the vial. In their original packaging, before use, when stored at -10 to -20°C, the unopened antibodies are intended for use with in-house enzyme-linked immunosorbent assay s for antibody. When present in the added material, tPA binds to the anti-human antibody. When present in the added material, tPA binds to the anti-human antibody.

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 tPA peroxidase conjugate, which reacts specifically with human tPA. Following another washing step, the peroxidase substrate, o-Phenylenediamine (OPD) in presence of hydrogen peroxide (H₂O₂), 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 tPA present in the tested sample.

REAGENTS:
Required Materials provided (enough for 5x96 Tests):
- Capture Antibody (TPA-EIA-C). 1 vial of 0.5 mL affinity purified polyclonal antibody specific for human TPA. For coating plates. Supplied in a 50% v/v glycerol solution. Yellow cap.
- 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.

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 for 2 hours @ 22°C or overnight @ 2-8°C.
2. Blocking: Empty contents of plate and add 150 µL of Blocking Solution to each well. Incubate plate @ 22°C for 90 minutes. Wash plate 3X with Wash Solution.
4. Detecting Antibody: Dilute the Detecting Antibody with Sample Diluent 1/100 and apply 100 µL to each well. Incubate plate @ 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 color to develop for 5-10 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 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.

Calibration Curve:
On bi-logarithmic graph paper, plot the known tPA concentrations on abscissa and the corresponding absorbance (A490) on ordinates to achieve standard plasmas with final tPA concentrations of 50, 25, 12.5, 6.25, 3.13 µM. Dilute standard plasmas and sample plasmas with Sample Diluent 1/4 and 1/8.

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

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