Version 17-05/25

User's Manual

Human tissue Plasminogen Activator Matched Antibody Pair

REF **ABPR-L033**

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 PLAT

General Description

Tissue-type plasminogen activator (tPA) is one of two major physiologic activators of plasminogen in plasma. It is a serine protease of 68 kDa produced primarily in endothelial cells but is also present in monocytes and megakaryocytes. Normal plasma tPA antigen concentrations have been reported from 20 ng/ml to 5 µg/ml, depending on the assay used, but typically most of the tPA (> 90%) is in complex with it's primary inhibitor, plasminogen activator inhibitor-1 (PAI-1). Structurally, tPA is a single-chain enzyme that consists of a catalytic domain followed by two kringle structures, an EGF domain and a finger domain. The activation of plasminogen by tPA is dependent on the presence of a fibrin cofactor. The binding of both tPA and plasminogen to fibrin is mediated in part through lysine binding sites within the kringle structures of both enzyme and substrate, but also through the finger domain of tPA. Activation of plasminogen by tPA occurs by cleavage after residue Arg560 to produce the two-chain active serine protease plasmin. The activity of tPA is regulated in part by a very short half life in circulation (t½ of ~4 minutes) and by circulating protease inhibitors PAI-1 and to a lesser extent α2 macroglobulin.

Principles of Testing

Affinity-purified antibody to tPA is coated onto the wells of a microtitre plate. Any remaining binding sites on the plastic wells are blocked with an excess of bovine serum albumin. The plates are washed and plasma or other fluids containing tPA are applied. The coated antibody will capture the tPA in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to tPA is added to the plate to bind to the captured tPA. 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 H2SO4 and the colour produced is quantified using a microplate reader. The colour generated is proportional to the concentration of tPA present in the sample.

Reagents And Materials Provided

1. Capture Antibody (yellow): 0.5 ml of polyclonal affinity purified anti-tPA antibody for coating plates.
2. Detecting Antibody (red): 0.5 ml of peroxidase conjugated polyclonal anti-tPA antibody for detection of captured tPA.

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 –10 to -20°C. Keep vials tightly capped. Do not store in frost-free freezers.

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(1%, 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 H2SO4
9. Other: Microplates; tPA reference standard; tPA/PAI-1 deficient plasma; Microplate washer; Mircoplate reader.

Storage

-10 to -20°C

Assay Procedure

1. **Coating of plates:** Dilute the capture antibody 1/100 in coating buffer(in a poly propylene tube) and immediately add 100 µl per well in the plate. Incubate for 2 hours at ambient temperature or overnight at 2-8°C.
2. **Blocking:** Empty contents of plate and add 150 µl of blocking buffer to every well and incubate for 60 minutes at 22°C. Wash plate 3 times with wash buffer.
3. **Preparation of tPA Reference Standards:** Reconstitute vials of tPA standard and tPA/PAI-1 deficient plasma according to manufacturers instructions. After reconstitution, dilute the tPA standard into tPA/PAI-1 deficient plasma to achieve six reference standard plasmas with final tPA concentrations of 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/ml respectively.
4. **Samples:** Reference plasmas prepared in step 3 and test plasmas are diluted 1/4 and 1/8 in HBS-BSA-T20 sample diluent. Samples should be run in duplicate. Apply 100 µl/well and incubate plate at 22°C for 90 minutes. Wash plate 3 times with wash buffer.
5. **Detecting Antibody:** Dilute the detecting antibody 1/100 in HBS-BSA-T20 sample diluent and apply 100 µl to each well. Incubate plate at 22°C for 90 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 5-10 minutes then stop colour reaction with the addition of 50 µl/well of 2.5 M H2SO4. The plate can be read at a wavelength of 490 nm.