Catalog # HTPAKT

Human tPA activity assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

This human tPA activity assay is intended for the quantitative determination of active tissue plasminogen activator in human plasma and other biological fluids.

BACKGROUND

Tissue plasminogen activator is a serine protease that catalyzes the activation of plasminogen to plasmin [1]. Clinical studies have indicated that high tPA levels may increase the risk for thrombosis [2], whereas decreased levels may cause neuronal plasticity and degeneration [3].

ASSAY PRINCIPLE

Functionally active tPA will form a covalent complex with the biotinylated human PAI-1 which is bound to the avidin on the plate. After appropriate washing steps, polyclonal anti-human tPA primary antibody binds to the captured tPA. Excess antibody is washed away and bound polyclonal antibody is then reacted with the secondary antibody conjugated to horseradish peroxidase. Following an additional washing step, TMB is used for color development at 450nm. The amount of color development is directly proportional to the concentration of active tPA in the sample.

DEFINITION OF tPA UNIT

Conversion Factor: 1 tPA IU = 1.45 ng

The tPA activity standard provided contains human single chain tPA and is calibrated against the International Standard for tPA, lot 98/714 distributed by NIBSC, South Mimms, Potters Bar, Hertfordshire, UK.

REAGENTS PROVIDED

- **Avidin Coated Plate:**
  1-96 well immulon strip plate coated with avidin, blocked, and dried
- **10X Wash Buffer:**
  1 bottle of 50mL wash; bring up to 1X using DI water
- **General Assay Diluent:**
  1 bottle of 10mL diluent
- **Biotinylated PAI-1:**
  1 vial of lyophilized biotinylated PAI-1
- **Human tPA activity standard:**
  1 vial of lyophilized standard
- **Anti-human tPA primary antibody:**
  1 vial of lyophilized polyclonal antibody
- **Anti-rabbit secondary antibody:**
  1 vial of concentrated horseradish peroxidase conjugated antibody
- **TMB substrate solution:**
  1 bottle of 10 ml solution

STORAGE AND STABILITY

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All

other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

REAGENTS AND EQUIPMENT REQUIRED

- 1-channel pipettes covering 0-10µl and 200-1000µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 50ml tubes
- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- TBS buffer
- 3% Blocking buffer
- Microtiter plate spectrophotometer operable at 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

WARNINGS

Warning - The tPA standards are of human origin. Each donor unit has been tested and found negative for the presence of HBsAg, anti-HIV 1+2, anti-HBc, and anti-HCV.
Since no tests are currently available to assure that no infectious agents are present, the standard must be treated as is recommended at the Biosafety Level 2 as potentially infectious human serum or blood specimen in the Centres for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories", 1984.
Warning – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

PRECAUTIONS

- **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris-HCl, 0.15M NaCl, pH 7.4
- **Blocking buffer:** 3% BSA in TBS buffer

SPECIMEN COLLECTION

Samples of human plasma in citrate or EDTA may be assayed with this kit. Plasma in heparin is not recommended. It is important to ensure a platelet free preparation as platelets can release PAI-1, which in turn could potentially form a complex with active tPA. Serum and cell culture media at neutral pH may also be used.
For best results, collect 9 volumes of blood in 1 volume of 0.1M acidified citrate, preferably using Stabilyte™ evacuated vials (Biopool, cat# 102080) [5]. The low pH of the resulting plasma insures that PAI-1 is inhibited from quenching tPA activity [6]. Immediately after collection of blood, samples must be centrifuges at 2500Xg for 15 minutes. The plasma must be transferred to a clean plastic tube and stored on ice prior to analysis. The tPA activity samples collected in the Stabilyte™ media are stable for up to 5 hours on ice, up to one month frozen at -20°C or up to 5 months frozen at -70°C.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Biotinylated Human PAI-1 Addition:
Remove microtiter plate from bag. Add 10ml of 3% BSA blocking buffer directly to the biotinylated human PAI-1 vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Preparation of Standard:
Reconstitute standard as directed on vial to give a 6.9 IU/mL standard solution.

Dilution table for preparation of human tPA standards:

tPA concentration (IU/mL)	µl of 6.9 IU/mL tPA standard	µl of 3% BSA Blocking Buffer
1	73	427
0.5	36	464
0.4	29	471
0.25	18	482
0.1	7	493
0.05	4	496
0	0	500

NOTE: DILUTIONS FOR THE STANDARD CURVE MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition:
If samples are at neutral pH, add 100µl of tPA standards (in duplicate) and unknowns to wells. If the pH of samples is below pH 6.0, first add 40µl of General Assay Diluent to all wells, then add 60µl of tPA standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.
NOTE: This assay measures active tPA in the 0-1 IU/mL range (0-1.45 ng/mL). If the unknown is thought to have high tPA levels, dilutions may be made in a similar biological fluid devoid of tPA, or in 3% BSA blocking buffer.

Primary Antibody Addition:
Add 10ml of 3% BSA blocking buffer directly to the primary antibody vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

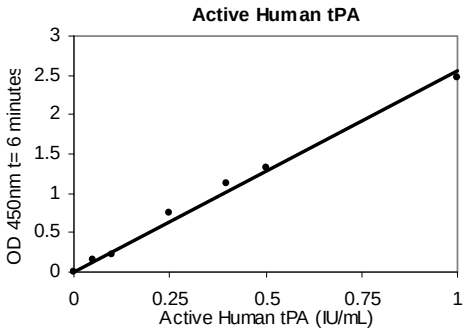
Secondary Antibody Addition:
Dilute 1µl of conjugated secondary antibody in 10ml of 3% BSA blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation:
Add 100µl of substrate solution to all wells and shake plate for 5-15 minutes. Quench the reaction with the addition of 50µl of 1N H_2SO_4 and read final absorbance values at 450nm.
NOTE: Time for substrate development is dependent on needs of researcher.

Measurement:
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm, A_{450}.

Assay Calibration:
Plot A_{450} against the amount of tPA in the standards. Fit a straight line through the points using a linear fit procedure. The tPA activity in the unknowns can be determined from this curve.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)



EXPECTED VALUES

The basal level of tPA in healthy humans was found to be between 0.2-2 IU/mL [8].

Abnormalities in tPA levels have been reported in the following conditions:

♦ Neuronal plasticity and degeneration: Decreased levels of tPA have been implicated in the process of neuronal plasticity and degeneration [1,3].

♦ Arthritis: Decreased tPA levels may exacerbate arthritis [4].

♦ Deep venous thrombosis: Increased tPA levels may contribute to deep venous thrombosis [2].

♦ Coronary heart disease: Increased tPA levels may contribute to severe coronary heart disease [2].

♦ Pregnancy: Increased tPA levels are observed during pregnancy [7].

PERFORMANCE CHARACTERISTICS

Sensitivity = 0.006 IU/ml
(calculated by determining the OD of 20 reps of So and 20 reps of the low standard)
Linearity
The slope = 0.9776
Correlation coefficient = 0.9971
Intra Assay Precision
High 3.8%, Medium 4.0%, Low 9.8%
(calculated by running 20 reps of each concentration in an assay)

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling or from contact with the above product.

REFERENCE

1. Sallés Fernando J, *et al*.: Localization and Regulation of the Tissue Plasminogen Activator – Plasmin System in the Hippocampus. The Journal of Neuroscience., March;**22(6)**: 2125-2134, 2002.
2. Wiman Björn, *et al*.: Inactivation of Tissue Plasminogen Activator in Plasma. The Journal of Biological Chemistry, March;**259(6)**: 3644-47, 1984.
3. Hastings Gregg A., *et al*.: Neuroserpin, a Brain-associated Inhibitor of Tissue Plasmiogen Activator Is Localized Primarily in Neurons. The Journal of Biological Chemistry, Dec;**272(52)**: 33062-33067, 1997.
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5. Ranby M, *et al*.: Blood Collection in Strong Acidic Citrate Anticoagulant used in a Study of Dietary Influence on Basal tPA Activity. Thromb Haemost, Nov24;**62(3)**: 917-22, 1989.
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7. Kruithof EK, *et al*.: Fibrinolysis in Pregnancy: a study of plasminogen activator inhibitors. Blood, Feb;**69(2)**: 460-466, 1987.
8. Eliasson, *et al*.: Influence of Gender, Age and Sampling Time on Plasma Fibrinolytic Variables and Fibrinogen. Fibrinolysis **7**: 316-323, 1993.