Rat tPA Activity Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

**INTENDED USE**
Rat tPA activity assay is intended for the quantitative determination of active tissue plasminogen activator in rat plasma.

**BACKGROUND**
Tissue plasminogen activator (tPA) is a serine protease that converts plasminogen to the active serine protease plasmin in the blood fibrinolytic system [1,2]. It also plays an important role in the removal of incipient thrombi [3]. tPA is widely used for the thrombolytic treatment of acute myocardial infarction [3].

**ASSAY PRINCIPLE**
Functionally active rat tPA will bind to the biotinylated human PAI-1 coated on the microtiter plate. Only free active enzyme will react with the PAI-1 on the plate. After appropriate washing steps, polyclonal anti-murine tPA primary antibody binds to the captured enzyme. Excess antibody is washed away and bound polyclonal antibody is then reacted with the secondary antibody conjugated to horseradish peroxidase. TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of tPA.

**REAGENTS PROVIDED**
- 8 uPA coated 12-well microtiter strips: containing Avidin dried and blocked on the strip well surface.
- **10X Wash Buffer:**
  - 1 bottle of 50ml wash; bring to 1X using DI water
- **Biotinylated PAI-1:** 1 vial lyophilized biotinylated PAI-1
- **Rat tPA activity standard:**
  - 1 vial Frozen
- **Anti-murine tPA primary antibody:**
  - 1 vial lyophilized polyclonal anti-mouse antibody
- **Anti-rabbit horseradish peroxidase conjugate secondary antibody:**
  - 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 10 ml

**STORAGE AND STABILITY**
Rat tPA activity standard must be stored at -70°C when not in use. All other reagents must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Kit should be used no later than the expiration date. **DO NOT** freeze/thaw the primary antibody more than once.

**REAGENTS AND EQUIPMENT REQUIRED**
- 1-channel pipettes covering 1-10µl, 20-200µl, 200-1000µl and 500-5000µl
- 12-channel pipette for 30-300µl
- Paper towels or kimwipes
- 1.5ml microcentrifuge tubes
- 1N H₂SO₄
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
• TBS buffer
• Blocking buffer
• Microtiter plate spectrophotometer operable at 450nm
• Microtiter plate shaker with uniform horizontally circular movement up to 300rpm

WARNING
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**: 0.10M TRIS, 0.15M NaCl, pH 7.4
• **Blocking buffer**: 3% BSA in TBS buffer

SPECIMEN COLLECTION
Collect 9 volumes of blood in 1 volume of 0.1M trisodium citrate or acidified citrate. Immediately after collection of blood, samples must be centrifuged at 3000Xg for 15 minutes. It is important to ensure a platelet free preparation as platelets can release PAI-1, which in turn could potentially form a complex with tPA. The plasma must be transferred to a clean plastic tube and must be stored on ice prior to analysis. The tPA activity samples are stable for up to 24 hours or stored at -20°C for up to one month and thawed three times without loss of tPA activity.

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 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:**
Prepare the tPA standard according to the dilution table insert found in the kit.

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

**Standard and Unknown Addition:**
Add 100µl standard in duplicate and unknown 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:** If the unknown is thought to have high tPA levels, dilutions may be made in 3% BSA blocking buffer.

**Primary Antibody Addition:**
Add 10ml 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 into 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 TMB substrate to all wells and shake plate for 3-5 minutes. Quench the reaction by the addition of 50µl of 1N H₂SO₄ and read final absorbance values at 450nm.

**Measurement:**
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm, A₄₅₀.

**Assay Calibration:**
Plot A₄₅₀ 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 by from this curve.

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

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**EXPECTED VALUES**

NOTE: Rat tPA concentrations: no specific data reported. Refer to references for mouse tPA.
The concentration level of tPA antigen in murine plasma has been reported to be 2.5+/-.1.0 ng/ml [4].

Abnormalities in tPA levels have been reported in the following condition:
♦ Venous Thrombosis: Locally applied tPa reduces thrombus formation after vascular injury [9].
♦ Ischemic Diseases: tPA may affect the course of ischemic diseases [5].
♦ Pathological Infarction: tPA may prevent or limit pathological infarction and improve neurological functions [6]. Usage of tPA at the onset of ischemic stroke improves clinical outcome [7].
♦ Blood-Brain Barrier: is necessary and sufficient to directly increase the vascular permeability in the early stages of BBB opening [8].

**QUALITY CONTROL**
The performance of each assay can be controlled using a positive quality control sample. An abnormally high tPA sample can be prepared by freezing aliquots of plasma known to contain a high level of tPA.
The assay measures active tPA in the 0.05-10 ng/ml range. Samples giving tPA levels above 10ng/ml should be diluted in plasma devoid of active tPA.

This information is believed to be correct but does not purport 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**