Catalog # MTPAKT

# Mouse tPA Activity Assay

Strip well format. Reagents for up to 96 tests.

## For Research Use Only.

### INTENDED USE

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

### BACKGROUND

Tissue plasminogen activator (tPA) is a serine protease that converts plasminogen to plasmin in the blood fibrinolytic system [1,2,3,9]. It also plays an important role in the nervous system, including the processes of neuronal migration, neurite outgrowth, and neuronal plasticity [1,2,4,7,10]. tPA has been suggested to have a role in several neuropathological conditions such as cerebral ischemia, seizures, and demyelinating diseases [1,3,5].

### ASSAY PRINCIPLE

Functionally active mouse 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. A standard calibration curve is prepared using dilutions of tPA along with the samples to be measured. After appropriate washing steps, monoclonal anti-murine tPA primary antibody binds to the captured enzyme. Excess antibody is washed away and bound monoclonal 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.

### REAGENTS PROVIDED

- **Avidin Coated Plate:**
  1-96 well immulon strip plate (8X12 removable strips) coated with avidin, blocked, and dried
- **10X Wash Buffer:**
  1 bottle of 50ml wash; bring to 1X using DI water
- **Biotinylated PAI-1:**
  1 vial of lyophilized biotinylated PAI-1
- **10X TBS Buffer pH 7.4:**
  1 vial of 5ml TBS buffer
- **Murine tPA activity standard:**
  1 vial of lyophilized standard
- **Anti-murine tPA primary antibody:**
  1 vial of lyophilized monoclonal antibody
- **Anti-mouse secondary antibody:**
  1 vial of concentrated HRP labeled 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 1-10µl, 20-200µl, 200-1000µl and 500-5000µl
- 12-channel pipette for 30-300µl
- Paper towels or kimwipes
- 1.5ml microcentrifuge tubes

- 1N $H_2SO_4$
- 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

## WARNINGS

**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 (BSA):** 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. Samples must be at a neutral pH to be used in the assay. If samples were collected in citrate pH should be brought up to neutral with the 10X TBS provided in the kit as described below.

## 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 **1,000ng/ml** standard stock solution. Prepare the tPA standard according to the following dilution table.

| tPA concentration (ng/ml) | Dilutions |
|---|---|
| 50 | 950µl (BSA) + 50µl (standard from vial) |
| 25 | 500µl (BSA) + 500µl (50ng/ml) |
| 10 | 600µl (BSA) + 400µl (25ng/ml) |
| 5 | 500µl (BSA) + 500µl (10ng/ml) |
| 2 | 600µl (BSA) + 400µl (5ng/ml) |
| 1 | 500µl (BSA) + 500µl (2ng/ml) |
| 0.5 | 500µl (BSA) + 500µl (1ng/ml) |
| 0.25 | 500µl (BSA) + 500µl (0.5ng/ml) |
| 0.1 | 600µl (BSA) + 400µl (0.25ng/ml) |
| 0.05 | 500µl (BSA) + 500µl (0.1ng/ml) |
| 0 | 500µl (BSA) Zero point to determine background |

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

**Standard and Unknown Addition:**

If using citrated plasma or samples with pH lower than 6.0 add 30µl of 10X TBS buffer to each well. If using samples at a neutral pH this step should be omitted. Add 100µl standards and unknowns to wells, in duplicates. 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:** Samples giving tPA levels above 50ng/ml should be diluted in plasma devoid of active tPA or 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 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 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N $H_2SO_4$ stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly and read final absorbance values at 450nm. For best results read plate immediately

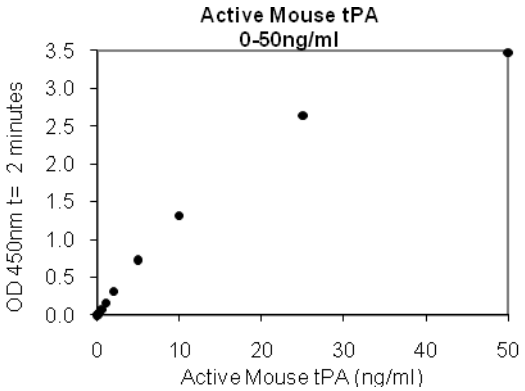
**Measurement:**

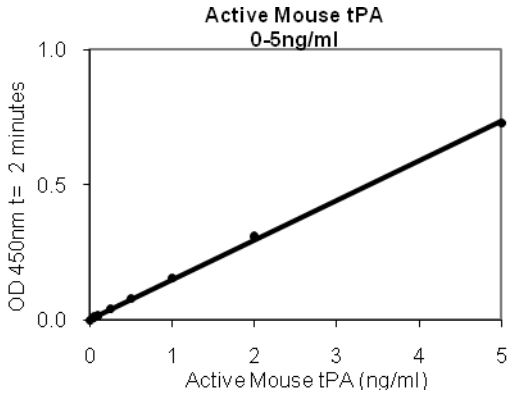
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance ($A_{450}$).

**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 by from this curve.

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




## EXPECTED VALUES

The concentration level of endogenous tPA antigen in murine plasma has been reported to be 2.5+/-1.0 ng/ml [15]. In house testing of pooled normal mouse plasma in citrate indicates tPA levels vary by mouse strain:

| Strain | Active tPA | Total tPA |
|---|---|---|
| NSA/CF-1 | 9.9 ng/ml | 9.4 ng/ml |
| C57BL6 | 1.4 ng/ml | 2.4 ng/ml |
| CD-1 | 0.4 ng/ml | 0.4 ng/ml |

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

♦ Venous Thrombosis: Endogenous tPA plays a key role in restoring cerebral blood flow and limiting infarct size after thrombosis [6].

♦ Spinal Cord Contusion: Suppression of tPA production may help decrease secondary injury after spinal cord injury [1].

♦ Ischemic Diseases: tPA may attenuate neuronal injury after mild focal cerebal ischemia [5]. tPA may be involved in the regulation of blood vessel tone, which may affect the course of ischemic diseases [3].

♦ Bone Formation: A decreased in tPA may result in an increase of bone formation [14].

♦ Diabetic Retinopathy: Increased tPA levels have been associated with proliferative diabetic retinopathy [8].

♦ Adipose Tissue Development: A decrease in tPA may increase the development of adipose tissue in diet-induced obesity [11].

♦ Stress-induced Anxiety: tPA is critical for the development of anxiety-like behavior after stress [12].

## PERFORMANCE CHARACTERISTICS

The assay measures active tPA in the 0.1-50 ng/ml range.

## 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

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| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0 | 0.05ng/ml | 0.1ng/ml | 0.25ng/ml | 0.5ng/ml | 1ng/ml | 2ng/ml | 5ng/ml | 10ng/ml | 20ng/ml | 50ng/ml | |
| B | 0 | 0.05ng/ml | 0.1ng/ml | 0.25ng/ml | 0.5ng/ml | 1ng/ml | 2ng/ml | 5ng/ml | 10ng/ml | 20ng/ml | 50ng/ml | |
| C | | | | | | | | | | | | |
| D | | | | | | | | | | | | |
| E | | | | | | | | | | | | |
| F | | | | | | | | | | | | |
| G | | | | | | | | | | | | |
| H | | | | | | | | | | | | |

**Sample Plate Layout: 96 Well Plate**
**Standards: 22 wells**
**Samples: 74 wells**