

Mouse tPA Total Antigen Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

Mouse tPA total antigen assay is intended for the quantitative determination of total tissue plasminogen activator in mouse plasma and other biological fluids.

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

Mouse tPA will bind to the capture antibody coated on the microtiter plate. Free and complexed enzyme will react with the capture antibody 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-mouse 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. TMB substrate is used for color development at 450nm. The amount of color development is directly proportional to the concentration of tPA antigen in the sample.

REAGENTS PROVIDED

- ◆ **Antibody Coated Plate:**
1-96 well immulon strip plate (8X12 removable strips) coated with capture antibody, blocked, and dried
- ◆ **10X Wash Buffer:**
1 bottle of 50ml wash; bring to 1X using DI water
- ◆ **Mouse tPA antigen standard:**
1 vial of lyophilized standard
- ◆ **Anti-mouse 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 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

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

Samples of plasma, serum, cell culture media, or other biological fluids may be applied directly to the plate.

ASSAY PROCEDURE

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

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 (5 ng/ml)
1	500µl (BSA) + 500µl (2 ng/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	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:

Add 100µl of 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: If the unknown is thought to have high tPA levels, dilutions may be made 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 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. Quench the reaction by the addition of 50µl of 1M H₂SO₄ and read final absorbance values at 450nm.

NOTE: Time for substrate development is dependent on needs of researcher.

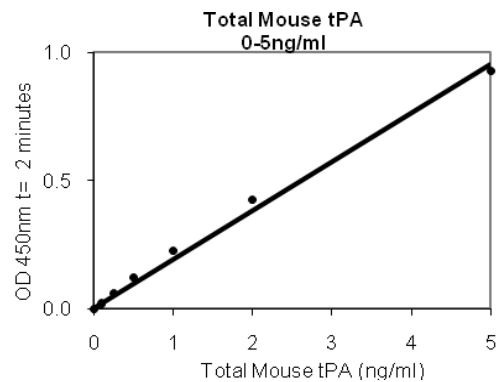
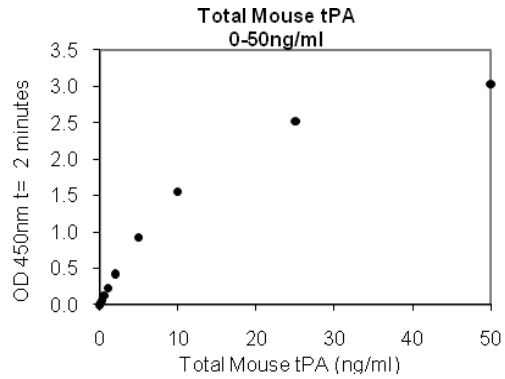
Measurement:

Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (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 antigen 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 tPA antigen in murine plasma has been reported to be 2.5+/-1.0 ng/ml [4]. 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: 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].

PERFORMANCE CHARACTERISTICS

The assay measures tPA antigen in the 0.1-50ng/ml range. Samples giving tPA levels above 50ng/ml should be diluted in plasma devoid of tPA or 3% BSA blocking buffer.

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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Example of Plate Layout

Standards: 20 wells Samples: 76 wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1ng/ml	0.25ng/ml	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml	25ng/ml	50 ng/ml		
B	0	0.1ng/ml	0.25ng/ml	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml	25ng/ml	50 ng/ml		
C												
D												
E												
F												
G												
H												