Mouse tPA Total Antigen Assay

Catalog No. IMTPAKT-TOT Lot No. 811

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

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 lyophilized standard

Anti-mouse tPA primary antibody:
1 vial lyophilized polyclonal anti-mouse antibody

Anti-mouse secondary antibody:
1 vial 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.

Required Reagents and Equipment

1-channel pipettes covering 0-10µL and 200-1000µL
12-channel pipette covering 30-300µL
Paper towels or kimwipes
50mL tubes, 1.5mL centrifuge tubes
1N H2SO4
DI water
Magnetic stirrer and stir-bars
Plastic containers with lids
Microtiter plate spectrophotometer operable at 450nm
Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

Warnings and Precautions

Warning – Avoid skin and eye contact when using TMB substrate solution. It may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.
•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.

Preperation of Reagents

TBS buffer: 0.10M TRIS, 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*

Standard and Unknown Addition:

Add 100ul 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 300ul 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 100ul to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300ul wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Assay Procedure Continued

Secondary Reagent 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 300ul wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe..

Substrate Incubation:

Add 100ul 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 50ul of 1N H2SO4 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

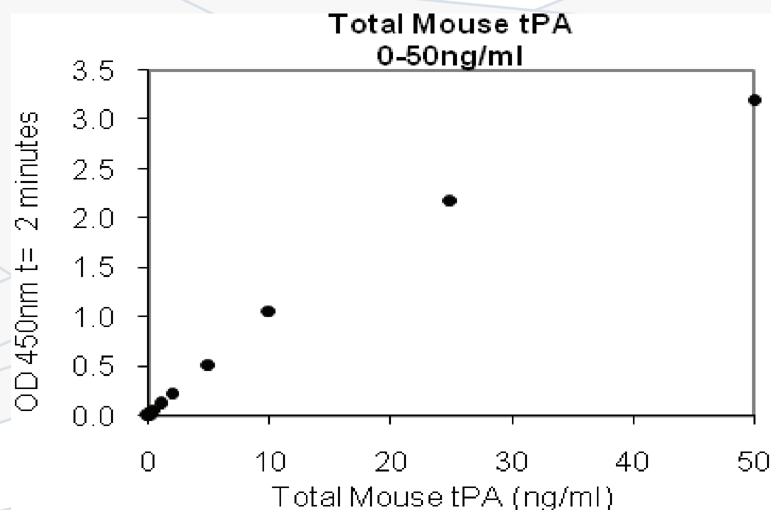
Measurement:

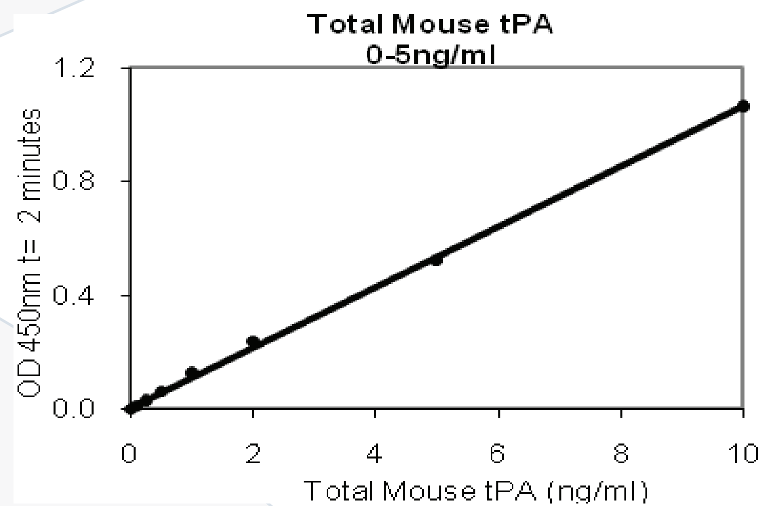
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A450).

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.

Standard Curve Examples





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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.