Enzyme Immunoassay for
Mouse Tissue Plasminogen Activator (tPA) Total Antigen

For Research Use Only

INTRODUCTION

The Mouse tPA total antigen assay is intended for the quantitative determination of total plasminogen activator antigen in biological fluids.

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].

PRINCIPLES OF PROCEDURE

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 450 nm.

MATERIALS PROVIDED

Component	Contents	Quantity	Storage	Cat. No.
Coated Plate	Capture antibody coated 96-well plate	1 plate	4°C	UP58a
Standard	Mouse tPA standard	1 vial	4°C	UP58b
Primary Antibody	Monoclonal anti-mouse tPA antibody	1 vial	4°C	UP58c
Secondary Antibody	Anti-mouse HRP conjugated antibody	1 vial	4°C	UP58d
Wash Buffer	10x solution for washing plate	50 mL	4°C	UP58e
Substrate	TMB substrate	10 mL	4°C	UP58f

MATERIALS NEEDED BUT NOT PROVIDED

1. Pipettes covering 0-10 μl and 200-1000 μl and tips
2. 12-channel pipette covering 30-300 μl
3. 1N H_2SO_4
4. DI water
5. Microtiter plate spectrophotometer with a 450 nm filter
6. Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm

STORAGE CONDITIONS

1. Store this kit and its components at 4°C until use.
2. 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.

PROCEDURAL NOTES

1. Use aseptic technique when opening and dispensing reagents.
2. This kit is designed to work properly as provided and instructed. Additions, deletions, or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.
3. Exercise universal precautions during the performance or handling of this kit or any component contained therein.

SAMPLE COLLECTION AND PREPARATION

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

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

REAGENT PREPARATION

1. **10x Wash Buffer:** Dilute to 1x by adding 450 mL of DI water to the 50 mL of 10x Wash Buffer prior to use.
2. **TBS Buffer (100 mL):** 0.1 M Tris-HCL, 0.15 M NaCl, pH 7.4
3. **3% BSA Blocking Buffer (20 mL):** 3% BSA (w/v) in TBS Buffer
4. **Standard:** Reconstitute with 1mL of the 3% BSA blocking buffer to obtain a 1000 ng/mL standard stock solution and vortex gently to mix. Prepare immediately prior to use.
5. **Primary Antibody:** Reconstitute with 10mL of 3% BSA Blocking Buffer and vortex gently to mix. Prepare immediately prior to use.
6. **Secondary Antibody:** Dilute 1uL with 10mL of 3% BSA Blocking Buffer and vortex gently to mix. Prepare immediately prior to use.

STANDARD PREPARATION

Reconstitute the Standard as directed on the vial to give a 100 ng/mL Standard Stock Solution. **Do not prepare the standards until you are ready to apply them to the plate.**

Table 1: Preparation of Standard Curve

Standard	tPA Concentration (ng/mL)	Blocking Buffer (µL)	Transfer Volume (µL)	Transfer Source	Final Volume (µL)
S_9	50	950	50	Stock Vial	500
S_8	25	500	500	S_9	600
S_7	10	600	400	S_8	500
S_6	5	500	500	S_7	600
S_5	2	600	400	S_6	500
S_4	1	500	500	S_5	500
S_3	0.5	500	500	S_4	500
S_2	0.25	500	500	S_3	600
S_1	0.1	600	400	S_2	1,000
B_0	0	500	---	---	500

ASSAY PROCEDURE

1. Add 100 μl of the Standards and unknowns to the wells in duplicate. Shake the plate at 300 rpm for 30 minutes at room temperature (RT). See Scheme 1 below for a sample plate layout.
2. Wash the plate three times with 300 μL of Wash Buffer. Remove excess Wash Buffer by gently tapping the plate on a paper towel.
3. Add 100 µl of the Primary Antibody to each well. Shake the plate at 300 rpm for 30 minutes at RT.
4. Wash the plate three times with 300 μL of Wash Buffer as in step 2.
5. Add 100 µl of the Primary Antibody to each well. Shake the plate at 300 rpm for 30 minutes at RT.
6. Wash the plate three times with 300 μL of Wash Buffer as in step 2.
7. Add 100 μl of TMB Substrate to each well. Shake the plate at 300 rpm for 2-10 minutes at RT.
8. Stop the reaction by adding 50 μl of 1N H_2SO_4 and read the plate at 450 nm.

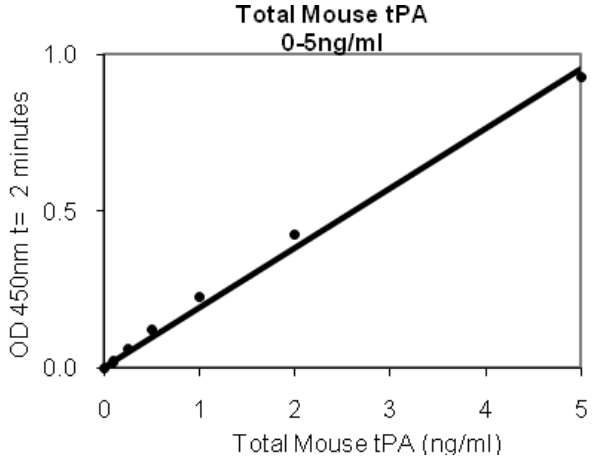
Scheme 1: Sample Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_9	S_8	S_7	S_6	S_5	S_4	S_3	S_2	S_1	B_0	U_1	U_2
B	S_9	S_8	S_7	S_6	S_5	S_4	S_3	S_2	S_1	B_0	U_1	U_2
C	U_3	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}	U_{14}
D	U_3	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}	U_{14}
E	U_{15}	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}	U_{26}
F	U_{15}	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}	U_{26}
G	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}	U_{38}
H	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}	U_{38}

CALCULATIONS

1. Average all the duplicate wells and subtract the zero point standard (B_0) from all standards and unknowns to obtain the corrected absorbance.
2. Plot the A_{450} against the concentration of tPA in the standards.
3. Fit a straight line through the points using a linear fit procedure.
4. Calculate the uPA concentrations in the unknowns using the standard curve. See Figure 1 bellow for an example of a typical standard curve.

Figure 1: Typical Standard Curve



EXPECTED VALUES

The concentration level of tPA antigen in mouse 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 conditions:

- 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].

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