Rat Tissue-type Plasminogen Activator (tPA) Total Antigen ELISA

For Research Use Only

INTRODUCTION

Tissue-type Plasminogen Activator (tPA) is a member of the serine proteinase family. tPA functions to lyse fibrin clots into soluble plasmin fragments. tPA is active in two forms, single chain and two chain. The two-chain tPA is created via interaction with the plasmin product cleaving the single chain. This two-chain form is regarded as the more active form.

Both single chain and two-chain tPA are complexable with PAI-1. PAI-1 acts as an inhibitor for tPA by binding to the tPA and thus stifling its ability to lyse fibrin.

tPA can serve as an indicator of both myocardial infarction for patients with impaired fibrinolytic systems as well as a marker for type-II diabetes.

PRINCIPLES OF PROCEDURE

This is an ELISA (Enzyme-Linked Immunosorbent Assay) for the quantitative analysis of tPa levels in biological fluid. This test kit operates on the basis of sandwich ELISA where free, latent and complexed tPa is quantified with the use of an HRP labeled secondary antibody.
The various forms of tPA present in the standard or unknown is captured by the tPA capture antibody coated on the well. A primary antibody specific for tPA is then added to each well followed by the HRP conjugated secondary antibody. The bound conjugated secondary antibody is detected by the addition of substrate, which generates an optimal color after 10 minutes. Quantitative test results may be obtained by the measure and comparison of the sample and standard absorbance readings when read with a microplate reader at 450 nm.

MATERIALS PROVIDED

Component	Contents	Quantity	Storage	Cat. No.
Coated Plate	Mouse anti-tPA coated 96-well microplate.	1 Plate	4°C	PA94a
Rat tPA Standard	Lyophilized rat tPA standard.	1 vial	4°C	PA94b
10x Wash Buffer	10x concentrated buffer used to wash the plate.	50 mL	4°C	PA94c
Primary Antibody	Lyophilized biotinylated polyclonal anti-mouse antibody.	1 vial	4°C	PA94d
Secondary Antibody	Concentrated avidin-HRP conjugate	1 vial	4°C	PA94e
TMB Substrate	TMB substrate used for color development.	10 mL	4°C	PA94f

MATERIALS NEEDED BUT NOT PROVIDED

1. Precision pipettes covering 10-1000 μl and tips
2. 1 N H_2SO_4
3. DI water
4. Microplate spectrophotometer with a 450 nm filter
5. Microplate 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. The reconstituted Standard and Primary Antibody may be aliquoted and stored at -70°C for later use. **DO NOT** freeze/thaw the Standard or 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

Collect 9 volumes of blood in 1 volume of 0.1 M trisodium citrate or acidified citrate. Immediately after collection of blood, samples must be centrifuged at 3000 x *g* 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 samples collected are stable for up to 24 hours at 4°C or stored at –20°C for up to one month. The samples can be thawed three times without loss of tPA activity.

REAGENT PREPARATION

1. **10x Wash Buffer:** Dilute the 50 mL of concentrate to 1x with 450 mL of DI water prior to use.
2. **TBS Buffer:** 0.1 M Tris, 0.15 M NaCl, pH 7.4.
3. **3% BSA Blocking Buffer:** 3% BSA in TBS Buffer.
4. **Primary Antibody:** Reconstitute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use.
5. **Secondary Antibody:** Dilute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use.

SANDARD PREPARATION

Reconstitute the Standard as directed on the vial to give a 1000 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
S_0	0	500	-	-	500

ASSAY PROCEDURE

1. Add 100 μl of the Standards and unknowns to the wells in duplicate. For a suggested plate layout, see **Scheme I** below. Shake the plate at 300 rpm for 30 minutes at RT.
2. Wash the plate 3 times according to the following wash procedure:
   a. Remove the contents of each well by inversion of the plate.
   b. Add 300 µL of 1x Wash Buffer to each well.
   c. Let stand for 2-3 minutes.
   d. Repeat procedure two more times, then proceed to step "e".
   e. Remove the contents of each well by inversion of plate into an appropriate disposal device.
   f. Tap out the remaining contents of the plate onto a lint free paper towel, then proceed to step 3.
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 as in step 2.
5. Add 100 µl of the Secondary Antibody to each well. Shake the plate at 300 rpm for 30 minutes at RT.
6. Wash the plate three times as in step 2.
7. Add 100 μl of TMB Substrate to each well. Shake the plate at 300 rpm for 10 minutes at RT.
8. Stop the reaction by adding 50 μl of 1N H_2SO_4 to each well and read the plate at 450 nm.

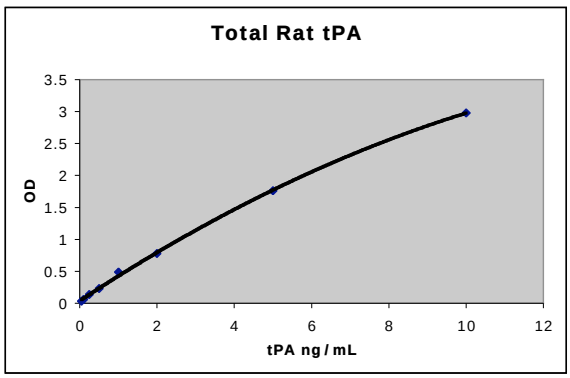
Scheme I:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_0	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	U_1	U_2
B	S_0	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	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}	BLK
H	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}	BLK

CALCULATIONS

1. Average the O.D. values for each pair of duplicate wells.
2. Subtract the average O.D. value of the blank wells (BLK) from all other pairs of wells. Some microplate readers can be programmed to do these subtractions automatically when reading the plate.
3. Plot a standard curve using the average O.D. value for each standard value versus the standard concentration.
4. Use the standard curve to determine the concentration of each unknown.

Figure 1: Typical Standard Curve



PERFORMANCE CHARACTERISTICS

Assay Range: 0.1-50 ng/mL

Samples with tPa levels higher than 50 ng/mL should be diluted in plasma devoid of active tPa.

EXPECTED VALUES

Note: No specific data has been reported for rat tPA concentrations. Refer to references for mouse tPA.

The concentration level of tPA antigen in mouse plasma has been reported to be 2.5+/-1.0 ng/ml (4).

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: tPA is necessary and sufficient to directly increase the vascular permeability in the early stages of BBB opening (8).

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