Total Human tPA Assay

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
Coated Plate	Anit-tPA coated 96-well plate	1 plate	4°C
Standard	Human tPA activity standard	1 vial	4°C
Primary Antibody	Anti-Human tPA antibody (lyophilized)	1 vial	4°C
Wash Buffer	10x solution for washing plate	50 mL	4°C
Substrate	TMB Substrate	10 mL	4°C
Secondary Antibody	Anti-rabbit HRP conjugated antibody	1 vial	4°C
Depleted Plasma	Human PAI-1/tPA depleted plasma	3 vials	4°C

MATERIALS NEEDED BUT NOT PROVIDED

1. 1 N H_2SO_4
2. TBS Buffer (see Reagent Preparation)
3. Blocking Buffer (see Reagent Preparation)
4. DI Water
5. Microplate reader with 450 nm filter
6. Microplate shaker with uniform horizontal circular movement up to 300 rpm

7. Precision pipettes that range from 10 µL-1000 µL and disposable tips

STORAGE

1. Store the kit and all of its components at 4°C before use.
2. If not using the entire plate at once, prepare only the appropriate amount of Primary Antibody and PAI-1 Standard. The remaining stock solutions should be frozen and stored at –70°C. Primary Antibody should be used within two weeks. All other components should remain refrigerated.
3. Store unused portions of the microplate in a pouch with a desiccant at 4°C.

WARNINGS AND PRECAUTIONS

WARNING: The tPA standard component and the depleted plasma is of human origin. Each donor unit has been tested and found negative for the presence of HbsAG, anti-HIV 1+2, anti-Hbc and anti-HCV.

Despite efforts to ensure safety, the tPA standard component should be treated as a Biosafety Level 2 and potentially infectious human blood specimen as directed in the Centers for Disease Control/ National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories" 1984.

1. 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.
2. Exercise universal precautions during the performance or handling of this kit or any component contained therein.

PROCEDURAL NOTES

1. This assay should be run at room temperature.
2. Use aseptic technique when opening and dispensing reagents.
3. To minimize error due to handling, wipe the exterior bottom of the microplate wells with a lint-free paper towel.

SAMPLE COLLECTION, STORAGE, AND PREPARATION

Samples should be collected using trisodium citrate, acidified citrate or Stabilite^{tm} (DiaPharma) collection media. Collection should be in accordance with the collection vials manufacturers instructions or in a 1:10 ratio of collection media to blood.

Immediately, upon collection of blood, the samples should be centrifuged at 3000 x g. This should ensure the removal of platelets as they can release PAI-1 that in turn complexes with uPA. The plasma can be transferred to a clean plastic tube and stored frozen for up to one month. Samples are stable for approximately 5 hours when stored at 4° C with the Sabilyte^{tm} media.

Note: Detergents such as Triton X cause interference with the assay. If using detergent extracted samples, it is necessary to dialyze the samples overnight to remove the detergent.

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 10 mL of 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use.
5. **Secondary Antibody:** Dilute with 10 mL of 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use.

STANDARD PREPARATION

The standard curve can be made in either 3% BSA Blocking Buffer or Depleted Plasma. If your samples are serum or plasma, the Depleted Plasma should be reconstituted at 1.0 mL per vial and used as the diluent. All other samples should use 3% BSA Blocking Buffer as the diluent

To prepare standard, reconstitute with 1.0 mL of 3% BSA Blocking Buffer or Depleted Plasma depending on your sample type. Prepare an initial dilution by adding 50 uL of 1,000 ng/mL stock with 150 uL of diluent.

Standard	tPA Concentration (ng/mL)	Amount of Diluent (µL)	Transfer Volume (µL)	Transfer Source	Final Volume (µL)
S_7	25	450	50	250 ng/mL Stock	300
S_6	10	300	200	S_7	250
S_5	5	250	250	S_6	300
S_4	2	300	200	S_5	250
S_3	1	250	250	S_4	250
S_2	0.5	250	250	S_3	300
S_1	0.2	300	200	S_2	500
S_0	0.0	250	---	---	250

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 room temperature (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. Tap out the remaining contents of the plate onto a lint free paper towel.
   c. Add 300 μL of 1x Wash Buffer.
   d. Let stand for 2-3 minutes.
   e. Repeat procedure two more times, then proceed to step “f”.
   f. Remove the contents of each well by inversion of plate into an appropriate disposal device.
   g. 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 300rpm 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 10-20 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.

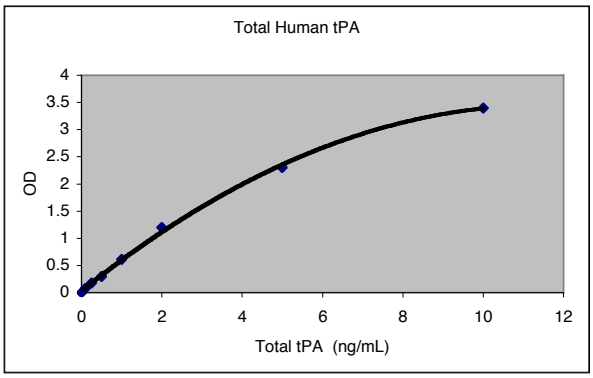
Scheme I:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_7	S_7	U_1	U_1	U_9	U_9	U_{17}	U_{17}	U_{25}	U_{25}	U_{33}	U_{33}
B	S_6	S_6	U_2	U_2	U_{10}	U_{10}	U_{18}	U_{18}	U_{26}	U_{26}	U_{34}	U_{34}
C	S_5	S_5	U_3	U_3	U_{11}	U_{11}	U_{19}	U_{19}	U_{27}	U_{27}	U_{35}	U_{35}
D	S_4	S_4	U_4	U_4	U_{12}	U_{12}	U_{20}	U_{20}	U_{28}	U_{28}	U_{36}	U_{36}
E	S_3	S_3	U_5	U_5	U_{13}	U_{13}	U_{21}	U_{21}	U_{29}	U_{29}	U_{37}	U_{37}
F	S_2	S_2	U_6	U_6	U_{14}	U_{14}	U_{22}	U_{22}	U_{30}	U_{30}	U_{38}	U_{38}
G	S_1	S_1	U_7	U_7	U_{15}	U_{15}	U_{23}	U_{23}	U_{31}	U_{31}	U_{39}	U_{39}
H	B_0	B_0	U_8	U_8	U_{16}	U_{16}	U_{24}	U_{24}	U_{32}	U_{32}	U_{40}	U_{40}

CALCULATIONS

1. Plot the A_{450} against the concentration of uPA in the standards.
2. Fit a straight line through the points using a linear fit procedure.
3. Calculate the uPA concentrations in the unknowns using the equation generated by the standard curve.

Typical Standard Curve:



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