tPA Activity ELISA

Catalog Number: TPA39-K01
96 Wells
For Research Use Only
v. 1.0

EAGLE BIOSCIENCES, INC.
20A NW Blvd., Suite 112, Nashua, NH 03063
Phone: 866-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM

DESCRIPTION

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.

PRINCIPLE OF ASSAY

This tPA Activity ELISA (Enzyme-Linked Immunosorbent Assay) is for the quantitative analysis of active tPA levels in biological fluid. This test kit operates on the basis of sandwich ELISA where free, active, tPA enzyme complexes with PAI-1 and is quantified with the use of an HRP labeled secondary antibody.

First the biotinylated PAI-1 binds to the avidin coated wells. Next, active tPA present in the standard or unknown, complexes with PAI-1. Inactive or complexed tPA is removed in a subsequent wash step. 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

1. **Biotinylated PAI-1:** 1 vial of lyophilized, biotinylated PAI-1.
2. **Human tPA Activity Standard:** 1 vial.
3. **Substrate:** 10 mL of ready to use TMB substrate.
4. **Anti-Human tPA Primary Antibody:** 1 vial of anti-human tPA antibody.
5. **HRP Secondary Antibody:** 1 vial of HRP conjugated secondary antibody.
6. **Coated Plate:** A 96 well microplate with avidin precoated on each well. The plate is ready for use as is. **DO NOT WASH!**
7. **10x Wash Buffer:** 50 mL of 10x wash solution – dilute to 1x prior to use.
8. **EIA Buffer:** 10 mL of assay buffer.

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. Beakers, flasks, cylinders, etc. required for preparation of reagents.
8. Precision pipettes that range from 10 µL-1000 µL and disposable tips.
9. Plastic film or plate cover to cover plate during incubation.

WARNINGS AND PRECAUTIONS

1. **DO NOT** use components beyond expiration date.
2. **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.
3. **DO NOT** pipette reagents by mouth.
4. Always pour substrate out of the bottle into a clean test tube - **DO NOT** pipette out of the bottle (if your tip is unclean you could contaminate your substrate).
5. All specimens should be considered potentially infectious. Exercise proper handling precautions.
6. **DO NOT** smoke, eat or drink in areas where specimens or reagents are being handled.
7. Use aseptic technique when opening and removing reagents from vials and bottles.
8. Keep plate covered except when adding reagents, washing or reading.
9. Kit components should be stored as instructed when not in use.

PROCEDURAL NOTES

1. Always use new pipette tips for the buffer, conjugate, standards, samples etc.
2. Before pipetting a reagent, rinse the pipette tip three times with that reagent (i.e. fill the tip with the desired amount of reagent and dispense back into the same vial

- repeat 2 times). Now the tip is properly rinsed and ready to dispense the reagent into your well or test tube.

3. When pipetting into the wells, DO NOT allow the pipette tip to touch the inside of the well, or any of the reagents already in the well - this can cause cross contamination.
4. Standards and samples should be assayed in duplicate.
5. To quantitate, always run a standard curve when testing samples.
6. Gently mix specimens and reagents before use. Avoid vigorous agitation.
7. Before taking an absorbance reading, wipe the outside bottom of the wells with a lint-free wiper to remove dust and fingerprints.
8. Desiccant bag must remain in foil pouch with unused strips. Keep pouch sealed when not in use to maintain a dry environment. Seal with a heat sealer. If a heat sealer is not available, thoroughly close the open end with tape. Try to remove excess air before sealing.
9. If not using the entire plate, prepare only the appropriate amount of primary and secondary antibody as-well-as the tPA standard and biotinylated PAI-1. The remaining stock solutions should then be refrozen and stored at –70 °C. All other components should remain refrigerated.
10. Reconstituted biotinylated PAI-1 must be used within two weeks. Refrigerate once reconstituted - do not freeze.

SAMPLE 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 manufacturer's 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

The following solutions should be prepared fresh before starting the assay.

-TBS Buffer: 0.10 M TRIS, 0.15 M NaCl, pH 7.4
-Blocking Buffer: 3% BSA in TBS buffer.

TEST PROCEDURES

Note: This assay should be performed at room temperature.

1. Remove microplate from the bag.
2. Add the indicated amount of BSA blocking buffer solution directly to the Biotinylated PAI-1 vial and slightly agitate until completely dissolved.
3. Add 100 µL of the BSA/Biotinylated PAI-1 mixture to the both standard and test wells.
4. Shake the plate at 300 rpm on a plate shaker for 30 minutes.
5. Wash wells according to the following wash procedure:
   a. Remove contents of the plate by inversion into an appropriate disposal device.
   b. Tap out remaining contents of the plate onto a lint free paper towel.
   c. Add 300 µL of wash buffer.
   d. Let stand for 2-3 minutes.
   e. Repeat procedure 2 more times then proceed to step "f".
   f. Remove contents of the plate by inversion into an appropriate disposal device.
   g. Tap out the remaining contents of the plate onto a lint free paper towel then proceed to step 6.

   Note: The decanted wells should be void of visible moisture before proceeding. If moisture is still visible then follow step "g" until satisfactory results are obtained.
6. Prepare standards as indicated in the provided dilution table.

   Note: The standards should be applied to the plate immediately upon preparation.
7. Add 40 µL of EIA buffer to the wells of the plate followed by 60 µL of standards or unknowns. See **Scheme I** for suggested template design.
8. Shake plate at 300 rpm on the plate shaker for 30 minutes.
9. Wash wells according to step 5 located above in this section.
10. Add the indicated volume of the 3% BSA blocking buffer directly to the Anti-Human tPA primary antibody and slightly agitate until completely dissolved.
11. Add 100 µL of the reconstituted Anti-Human tPA Primary Antibody to each well.

12. Shake plate at 300 rpm on the plate shaker for 30 minutes.
13. Wash wells according to step 5 located above in this section.
14. Make a stock concentration of secondary antibody by adding the indicated amount of secondary antibody stock solution to 10 mL of 3% BSA blocking buffer for a working concentration.
15. Add 100 µL of the working concentration BSA/ secondary antibody solution to each well.
16. Shake plate at 300 rpm on the plate shaker for 30 minutes.
17. Wash wells according to step 5 located above in this section.
18. Add 100 µL of TMB substrate to each well and allow to incubate for 10 minutes.
19. Quench reaction with 50 µL per well of 1 N H_2SO_4 and read plate at 450 nm.
20. If accounting for substrate background, use 2 to 8 wells as blanks with only substrate in the wells (150 µL/well). Subtract the average of these absorbance values from the absorbance values of the wells being assayed.

NOTE: Some microplate readers can be programmed to do these subtractions automatically when reading the plate. Consult your instrument manual.

Scheme I

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	U_1	U_2	U_3	U_4
B	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	U_1	U_2	U_3	U_4
C	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}	U_{14}	U_{15}	U_{16}
D	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}	U_{14}	U_{15}	U_{16}
E	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}	U_{26}	U_{27}	U_{28}
F	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}	U_{26}	U_{27}	U_{28}
G	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}	U_{38}	U_{39}	BLK
H	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}	U_{38}	U_{39}	BLK

CALCULATIONS

1. Subtract the average O.D. value of the blank wells (BLK) from all other pairs of wells.
2. Average the O.D. values for each pair of duplicate wells.

3. Plot a standard curve using the average O.D. value for each standard value versus the concentration of standard.
4. Determine the concentration of each unknown by interpolation from the standard curve.

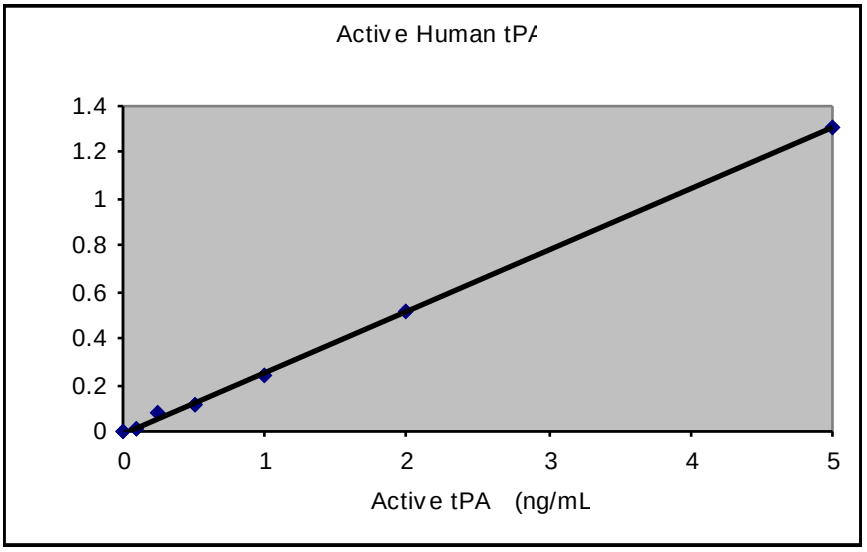
TYPICAL DATA

Note: "Typical data" is a representation. Variances in data will occur. Optical density readings may fluctuate during the shelf-life or due to lot variance.

Typical Data:

Standard	Standard Concentration (ng/mL)	Optical Density (Absorbance Value)
S_1	0.0	0.008
S_2	0.1	0.015
S_3	0.25	0.075
S_4	0.5	0.113
S_5	1	0.241
S_6	2	0.517
S_7	5	1.310

TYPICAL STANDARD CURVE



PERFORMANCE CHARACTERISTICS

Assay Range: 0.0- 5 ng/mL
Sensitivity: 0.006IU/mL

Samples with tPA levels higher than 5 ng/mL should be diluted in similar media devoid of active tPA.

REFERENCES

1. Thogersen A et al. (1998) Circulation 98: 2241-2247
2. Eliasson M et al. (2003) Cardiovascular Diabetology 2:19

For further information about this kit, its application or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

Product Developed and Manufactured in the USA