tPA Total Antigen ELISA

Catalog Number: TPN39-K01
96 Wells
For Research Use Only
v. 1.0

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DESCRIPTION

Tissue-type Plasminogen Actvator (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 Total Antigen ELISA (Enzyme-Linked Immunosorbent Assay) is 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

1. **Human tPA Activity Standard:** 1 vial.
2. **Substrate:** 10 mL. Stabilized 3,3', 5,5' Tetramethylbenzidine (TMB) plus Hydrogen Peroxide (H_2O_2) in a single bottle
3. **Anti-Human tPA Primary Antibody:** 1 vial of anti-human tPA antibody.
4. **HRP-Secondary Antibody:** 1 vial of HRP conjugated secondary antibody.
5. **10x Wash Buffer:** 50 mL of 10x wash solution – to be diluted prior to use.
6. **Coated Plate:** A 96 well microplate with tPA capture antibody precoated on each well. The plate is ready for use as is. **DO NOT WASH**
7. **Depleted Plasma:** Human PAI-1/tPA depleted Plasma (3 vials)

MATERIALS NEEDED BUT NOT PROVIDED

1. 1 N H2SO4.
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

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. **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 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 assay at once, then prepare the lyophilized tPA standard and primary antibody as directed and aliquot and store the remaining portions –70 °C. All other components should be stored at 4 °C.

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 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

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.
- **10x Wash Buffer**: Dilute the 50 mL of concentrate to 1x with 450 mL of DI water prior to use.
- **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.
- **Secondary 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.

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 µL of 1000 ng/mL stock with 150 µL of diluent.

Standard	tPA Concentration (ng/mL)	Amount of Diluent (µL)	Transfer Volume (µL)	Transfer Source	Final Volume (µL)
S7	25	450	50	250 ng/mL Stock	300
S6	10	300	200	S7	250
S5	5	250	250	S6	300
S4	2	300	200	S5	250
S3	1	250	250	S4	250
S2	0.5	250	250	S3	300
S1	0.2	300	200	S2	500
S0	0	250	n/a	n/a	250

ASSAY PROCEDURES

Note: This assay should be performed at room temperature.

1. Remove microplate from the bag.
2. Prepare standards as indicated in the provided dilution table.

   Note: The standards should be applied to the plate immediately upon preparation.

3. Add 100 µL of standards and unknowns to the plate.
4. Shake plate at 300 rpm on the 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.

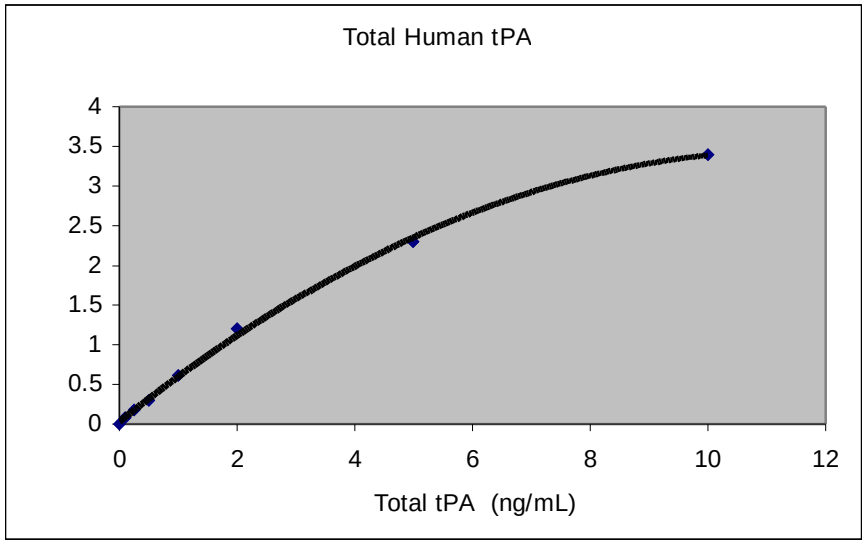
   Note: The decanted wells should be void of visible moisture before proceeding. If moisture is still visible then follow step "g" until satisfactory results are obtained.

6. Add 100 µL of the BSA/ Primary Antibody to each well.
7. Shake plate at 300 rpm on the plate shaker for 30 minutes.
8. Wash wells according to step 5 located above in this section.
9. Add 100 µL of the working concentration BSA/ Secondary Antibody solution to each well.
10. Shake plate at 300 rpm on the plate shaker for 30 minutes.
11. Wash wells according to step 5 located above in this section.
12. Add 100 µL of TMB substrate to each well. Shake the plate at 300 rpm for 30 minutes at RT.
13. Stop reaction with 50 µL per well of 1 N H_2SO_4 and read plate at 450 nm.

CALCULATIONS

1. Average the O.D. values for each pair of duplicate wells.
2. Plot a standard curve using the average O.D. value for each standard value versus the concentration of standard.
3. Determine the concentration of each unknown by interpolation from the standard curve.

TYPICAL STANDARD CURVE



PERFORMANCE CHARACTERISTICS

Assay Range: 0.0 - 10 ng/mL

Samples with uPA levels higher than 10 ng/mL should be diluted in similar media devoid of active uPA.

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	0.040
S_2	0.1	0.080
S_3	0.25	0.183
S_4	0.5	0.312
S_5	1	0.608
S_6	2	1.198
S_7	5	2.279
S_8	10	3.396

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