Package Insert

tPA Activity ELISA

Catalog Number: TPA39-K01
96 Wells
For Research Use Only
v. 3.0 (08.10.22)

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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. **Assay Buffer:** 10 mL of assay buffer used to neutralize acidic samples

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 of human plasma in citrate or EDTA may be assayed with this kit. Plasma in heparin is not recommended. It is important to ensure a platelet free preparation as

platelets can release PAI-1, which in turn could potentially form a complex with active tPA. Serum and cell culture media at neutral pH may also be used.

For best results, collect 9 volumes of blood in 1 volume of 0.1M acidified citrate, preferably using Stabilyte™ evacuated vials (Biopool, cat# 102080). The low pH of the resulting plasma insures that PAI1 is inhibited from quenching tPA activity. Immediately after collection of blood, samples must be centrifuged at 2500 x g for 15 minutes. The plasma must be transferred to a clean plastic tube and stored on ice prior to analysis. The tPA activity samples collected in the Stabilyte™ media are stable for up to 5 hours on ice, up to one month frozen at -20°C or up to 5 months frozen at -70°C. If sample pH is below 6.0 add 40 uL of assay buffer to all wells including the standard wells prior to adding samples to the plate.

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. **Biotinylated PAI-1**: Reconstitute with 10.0 mL of 3% BSA Blocking Buffer and vortex gently to mix. Prepare immediately prior to use.
5. **Standard**: Reconstitute with 10.0 mL of 3% BSA Blocking Buffer and vortex gently to mix. This will result in a 61 IU/mL standard solution. Further dilute 100 uL of this standard into 900 uL of 3% BSA Blocking Buffer to prepare a 6.1 IU/mL working dilution. Use the 6.1 IU/mL working dilution to prepare all standards as outlined in the table below. Prepare immediately prior to use.
6. **Primary Antibody**: Reconstitute with 10.0 mL of 3% BSA Blocking Buffer and vortex gently to mix. Prepare immediately prior to use.
7. **Secondary Antibody**: Briefly centrifuge the vial prior to opening. Dilute 2.0 uL with 10.0 mL of 3% BSA Blocking Buffer and vortex gently to mix. Prepare immediately prior to use.

STANDARD PREPARATION

1. Reconstitute the standard by adding 10 mL of blocking buffer directly to the vial and agitate gently to completely dissolve the contents. The concentration will be 5.3 IU/mL.
2. Use the dilution table below to prepare all standards for the assay.

tPA Concentration (IU/mL)	µl of 6.1 IU/mL tPA Standard	µL of Blocking Buffer	Total Volume
1	100	430	530
0.5	50	480	530
0.4	40	490	530
0.25	25	505	530
0.1	10	520	530
0.05	5	525	530

0.02	2	528	530
0.01	1	529	530
0	0	500	500

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. Add 100 µl of Standards and Samples to the wells in duplicate.

Note: The standards should be applied to the plate immediately upon preparation. If the pH of the Samples is lower than 6.0, add 40 µl of the Assay Buffer to the wells prior to adding the Standards or Samples. The step is unnecessary if the pH is neutral.

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 reconstituted Anti-Human tPA Primary Antibody 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 the working concentration BSA/ secondary antibody solution to each well.
13. Shake plate at 300 rpm on the plate shaker for 30 minutes.
14. Wash wells according to step 5 located above in this section.
15. Add 100 µL of TMB substrate to each well and shake plate at 300 rpm on the plate shaker for 3 - 10 minutes.
16. Stop reaction with 50 µL per well of 1 N H_2SO_4 and read plate at 450 nm.

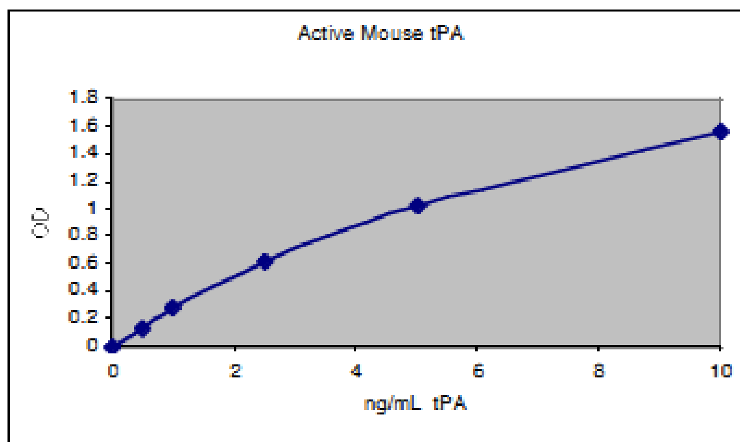
CALCULATIONS

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 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. The basal level of tPA in healthy humans was found to be between 0.2 – 2 IU/mL.

TYPICAL STANDARD CURVE



PERFORMANCE CHARACTERISTICS

- Assay Range: 0 - 1 IU/mL (0 – 1.77 ng/mL)
- Samples with tPA levels higher than 1 ng/mL should be diluted in similar media devoid of active tPA or 3% BSA Blocking Buffer.
- Sensitivity: 0.006 IU/ml (calculated by determining the OD of 20 reps of So and 20 reps of the low standard)
- Intra Assay Precision: High: 3.8%, Medium: 4.0%, Low: 9.8% (calculated by running 20 reps of each concentration in an assay)

REFERENCES

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

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