AssaySense Human tPA Chromogenic Activity Assay Kit

Catalog Number CT1001

Introduction

Tissue-type plasminogen activator (tPA) is a 68 kDa serine protease that converts the zymogen plasminogen into the active serine protease plasmin which digests fibrin and induce the dissolution of fibrin clots (1). tPA is synthesized by endothelial cells in normal blood vessels and displays relatively high affinity for fibrin, suggesting that it functions predominately in physiological thrombolysis *in vivo* (2). High level of tPA is a good prognostic marker for breast cancer (3). tPA may minimize the formation of metastasis by preventing tumor cell adherence at sites of trauma (4). On the other hand, gastrointestinal cancer is accompanied by a decrease in tPA (5).

Principle of Assay

The AssaySense Human tPA Chromogenic Activity Assay Kit is developed to determine human tPA activity in plasma and cell culture supernatants. The assay measures the ability of tPA to activate the plasminogen to plasmin in coupled or indirect assays that contain tPA, a fibrin analog, plasminogen, and a plsmin-specific synthetic substrate. The amount of plasmin produced is quantitated using a highly specific plasmin substrate releasing a yellow para-nitroaniline (pNA) chromophore. The change in absorbance of the pNA in the reaction solution at 405 nm is directly proportional to the tPA enzymatic activity.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- All human source materials have been tested and found to be negative to HbsAg, HIV-1 and HCV by FDA approved methods.

Reagents

The activity assay kit contains sufficient reagents to perform 100 tests using microplate method.

- **Microplate:** one 96 well polystyrene microplate (12 strips of 8 wells)
- **Assay Diluent:** 30 ml
- **Sample Diluent**: 30 ml
- **tPA Standard:** 1 vial human tPA (88 IU)
- **Human Fibriongen Fragments:** 1 vial
- **Human Plasminogen:** 1 vial
- **Plasmin Substrate:** 2 vials
- **Substrate Dilent:** 5 ml

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8^{0}C. Store reconstituted standard and reagents at -20^{0}C or below.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes (1-20 µl, 20-200 µl, and multiple channel)
- Deionized or distilled reagent grade water
- Incubator (37^{0}C)

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of acidified 0.5 M sodium citrate (pH 4.0) as an anticoagulant to prevent tPA-PAI complex formation. Centrifuge samples at 3,000x g for 15 minutes. Samples can be stored at < -70^{0}C. Avoid repeated freeze-thaw cycles. Prior to the analysis dilute samples (100 µl) 1:2 into Sample Diluent (100 µl) and incubate at room temperature for 10 minutes to overcome interference by plasmin inhibitors (6, 7).

- **Cell Culture Supernatants:** Centrifuge cell culture media at 3,000x g for 15 minutes at 4^{0}C to remove debris. Collect supernatants and assay. Samples can be store at < -70^{0}C. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- **Fibrinogen Fragments:** Add 1.2 ml Assay Diluent.
- **Plasminogen:** Add 1.2 ml Assay Diluent.
- **Plasmin Substrate:** Add 1.1 ml Substrate Diluent.
- **Standard Curve:** Reconstitute the tPA Standard with 2.2 ml of Sample Diluent to generate a solution of 40 IU/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (40 IU/ml) twofold with equal volume of Sample Diluent to produce 20, 10, 5, 2.5 and 1.25 IU/ml. Sample Diluent serves as the zero standard (0 IU/ml).

Standard Point	Dilution	[tPA] (IU/ml)
P1	1 part Standard	40.00
P2	1 part P1 + 1 part Sample Diluent	20.00
P3	1 part P2 + 1 part Sample Diluent	10.00
P4	1 part P3 + 1 part Sample Diluent	5.00
P5	1 part P4 + 1 part Sample Diluent	2.50
P6	1 part P5 + 1 part Sample Diluent	1.25
P7	Sample Diluent	0.00

Assay Procedure

- Assay Mix: Freshly prepare the desired volume of the Assay Mix by combining the following reagents according to the assay numbers (n).

Reagents	n=1
Assay Diluent	10 µl
Fibrinogen Fragments	10 µl
Plasminogen	10 µl

- Add 30 µl of the above Assay Mix to each well of the 96-well plate.
- Add 10 µl of tPA Standards or testing samples per well and mix gently. Incubate at 37^0C for 30 min.
- Add 20 µl of Plasmin Substrate to each well and mix gently. Incubate at 37^0C and read the absorbances at 405 nm periodically every 5 - 10 minutes up to one hour.

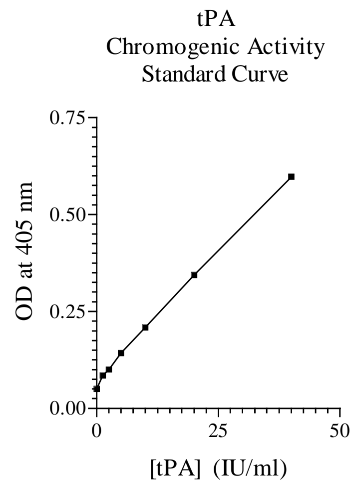
Assay Mix	30 µl
tPA or Samples	10 µl
37^0C, 30 minutes	
Plasmin Substrate	20 µl
37^0C, read the absorbances at 405 nm every 5 - 10 minutes for one hour	

Data Analysis

- Calculate the mean value of the triplicate for each standard and sample.
- To generate a Standard Curve from the initial reaction time, plot the graph using the standard concentrations on the x-axis and the corresponding mean 405 nm absorbance or change in absorbance per minute (ΔA/min) on the y-axis. The best-fit line can be determined by regression analysis of the linear portion of the curve.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor of 2.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of tPA is typically < 1 IU/ml.
- No significant cross-reactivity or interference was observed.

References

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