



AssaySense Human tPA Chromogenic Activity Kit

Catalog No. 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 induces 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). A 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 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, plasminogen, and a plasmin-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

- **Prepare all reagents (working diluent buffer, standards, substrate and plasminogen) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- 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)
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.

- **Assay Diluent:** 30 ml
- **tPA Standard:** 1 vial human tPA (48 IU)
- **Human Plasminogen:** 1 vial
- **Plasmin Substrate:** 2 vials

Storage Condition

- Store components of the kit at 2-8⁰C or -20⁰C upon arrival up to the expiration date.
- Store Plasminogen, Standard, and Plasmin Substrate at -20⁰C
- Store Microplate and Assay Diluent at 2-8⁰C
- Opened unused microplate wells may be returned to the pouch. Reseal along zip-seal.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes (1-20 μ l, 20-200 μ l, and multiple channel)
- Deionized or distilled reagent grade water
- Incubator (37⁰C)

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 3000 x g for 15 minutes. Prior to the analysis dilute samples 1:8 with Assay Diluent and incubate at room temperature for 10 minutes to overcome interference by plasmin inhibitors (6, 7). Samples can be stored at < -70⁰C. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x g for 15 minutes at 4⁰C to remove debris. Collect supernatants and assay. Samples can be store at < -70⁰C. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- **Standard Curve:** Reconstitute 48 IU of tPA Standard with 1.2 ml of Assay 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 duplicate or triplicate standard points by serially diluting the standard solution (40 IU/ml) 1:4 with Assay Diluent to produce 10, 2.5, 0.625, 0.156, and 0.039 IU/ml. Any remaining solution should be frozen at -20⁰C.

Standard Point	Dilution	[tPA] (IU/ml)
P1	1 part Standard (40 IU/ml)	40.00
P2	1 part P1 + 3 parts Assay Diluent	10.00
P3	1 part P2 + 3 parts Assay Diluent	2.500
P4	1 part P3 + 3 parts Assay Diluent	0.625
P5	1 part P4 + 3 parts Assay Diluent	0.156
P6	1 part P5 + 3 parts Assay Diluent	0.039
P7	Assay Diluent	0.000

- **Plasminogen:** Add 1.2 ml reagent grade water.
- **Plasmin Substrate:** Add 0.55 ml reagent grade water. Mix very well by gentle inversion. Wait at least 30 minutes before use (keep the vial on ice).

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (25⁰C) for specific sample binding and at 37⁰C for chromogenic activity assay. Seal the plate with sealing tape at each step.
- Remove excess microplate strips from the plate frame.
- Assay Mix: Freshly prepare the desired volume of the Assay Mix by combining the following reagents according to the assay numbers (n). It is recommended that Assay Mix be made in 10% excess.

<u>Reagents</u>	<u>n= 1</u>
Assay Diluent	50 µl
Plasminogen	10 µl
Plasmin Substrate	10 µl

- Add 80 µl of the above Assay Mix to each well.
- Add 20 µl of tPA Standard or samples per well and mix gently.
- Read the absorbance at 405 nm at zero minutes for background O.D. Seal the plate with sealing tape. Incubate the plate at 37⁰C in a humid incubator to avoid drying the plate.
- For HIGH tPA activity samples, read the absorbance at 405 nm every hour up to six hours.
- For LOW tPA activity samples, start to read the absorbance at 405 nm from 20 hours up to 26 hours.

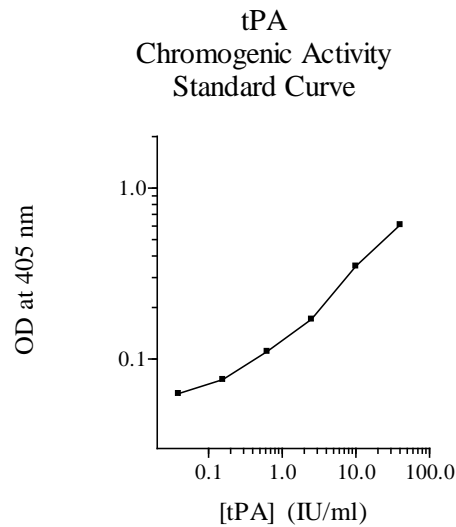
Assay Mix	80 µl
tPA Standard or Samples	20 µl
High tPA Activity Samples: incubate 37 ⁰ C, read the absorbance at 405 nm every hour for 6 hours.	
Low tPA Activity Samples: incubate 37 ⁰ C, read the absorbance at 405 nm every hour from 20 hours up to 26 hours.	

Data Analysis

- Calculate the mean value of the duplicate or 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 ($\Delta A/\text{min}$) 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.

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 ~ 0.03 IU/ml.
- No significant cross-reactivity or interference was observed.

References

- (1) Vassalli, J.D. *et al.* (1991) *J. Clin. Invest.* 88: 1067
- (2) Collen, D. and Lijnen, H.R. (1991) *Blood* 78:3114
- (3) Duffy, M.J. *et al.* (1992) *Fibrinolysis* 6: 55
- (4) Murthy, M.S. *et al.* (1991) *Cancer* 68: 1724
- (5) Nishino, N. *et al.* (1988) *Thromb. Res.* 50: 527

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