



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 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

- 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 unopened kit at 2-8⁰C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8⁰C. Store reconstituted standard and reagents at -20⁰C or below.

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. Samples can be stored at < -70⁰C. Avoid repeated freeze-thaw cycles. Prior to the analysis dilute samples (100 μ l) 1:2 into Assay Diluent (100 μ l) and incubate at room temperature for 10 minutes to overcome interference by plasmin inhibitors (6, 7).
- **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

- **Plasminogen:** Add 1.2 ml reagent grade water.
- **Plasmin Substrate:** Add 1.1 ml reagent grade water.
- **Standard Curve:** Reconstitute the tPA Standard with 1.2 ml of reagent grade water 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) 1:4 with Assay Diluent to produce 10, 2.5, 0.625, 0.156, and 0.039 IU/ml.

Standard Point	Dilution	[tPA] (IU/ml)
P1	1 part Standard (40 IU/ml)	40.000
P2	1 part P1 + 3 part Assay Diluent	10.000
P3	1 part P2 + 3 part Assay Diluent	2.500
P4	1 part P3 + 3 part Assay Diluent	0.625
P5	1 part P4 + 3 part Assay Diluent	0.156
P6	1 part P5 + 3 part Assay Diluent	0.039
P7	Assay Diluent	0.000

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	50 μ l
Plasminogen	10 μ l
Plasmin Substrate	20 μ l

- Add 80 μ l of the above Assay Mix to each well of the 96-well plate.
- Add 20 μ l of tPA Standards or samples per well and mix gently. Seal the plate with sealing tape. Incubate the plate at 37°C in a humid incubator to avoid drying the plate. For HIGH tPA activity samples, read the absorbances at 405 nm periodically every hour up to six hours. For LOW tPA activity samples, start to read the absorbances at 405 nm from 14 hours up to 21 hours.

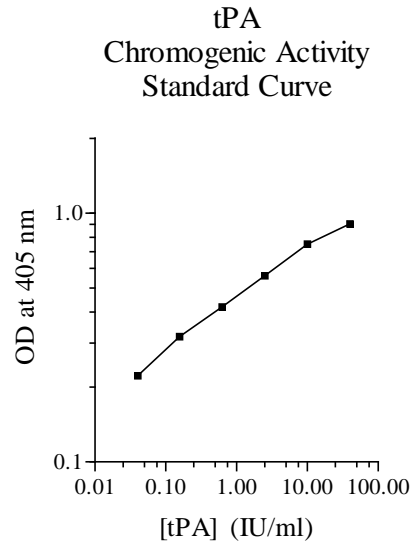
Assay Mix	80 μ l
tPA or Samples	20 μ l
37°C, read the absorbances at 405 nm every hour until 21 hours	

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 ($\Delta A/\text{min}$) 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 < 0.08 IU/ml.
- No significant cross-reactivity or interference was observed.

References

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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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