



AssaySense Human tPA Immuno-Chromogenic Activity Kit

Catalog No. CT2002

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 Immuno-Chromogenic Activity Assay Kit is developed to determine human tPA activity in plasma, serum and cell culture supernatants. The assay couples immunofunctional and indirect amidolytic assay. A polyclonal antibody specific for human tPA has been pre-coated onto a microplate and tPA is bound to the immobilized antibody. The assay measures the ability of tPA to activate the plasminogen to plasmin in direct assay 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 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.

- **tPA Microplate:** one 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human tPA.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human tPA Standard:** 1 vial, lyophilized (16 IU).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml).

- **Assay Diluent:** 1x, 30 ml.
- **Plasminogen:** 1 vial, lyophilized.
- **Plasmin Substrate:** 2 vials, lyophilized.

Storage Condition

- Store components of the kit at 2-8⁰C or -20⁰C upon arrival up to the expiration date.
- Store tPA Standard, Plasminogen, and Plasmin Substrate at -20⁰C
- Store Microplate, Diluent, and Wash Buffer at 2-8⁰C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Opened Diluent may be stored for up to 1 month at 2-8⁰C.

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 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and assay. Store undiluted samples at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Collect cell culture media and centrifuge at 3000 x g for 10 minutes at 4⁰C to remove debris and assay. Samples can be stored at < -20⁰C. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **Standard Curve:** Reconstitute the tPA Standard with 4 ml of Assay Diluent to generate a stock solution of 4 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 (4 IU/ml) 1:2 with Assay Diluent to produce 2, 1, 0.5, and 0.25 IU/ml. Assay Diluent serves as the zero standard (0 IU/ml). Any remaining solution should be frozen at -20⁰C.

Standard Point	Dilution	[FVIIa] (IU/ml)
P1	1 part Standard (4 IU/ml)	4.000
P2	1 part P1 + 1 part Assay Diluent	2.000
P3	1 part P2 + 1 part Assay Diluent	1.000
P4	1 part P3 + 1 part Assay Diluent	0.500
P5	1 part P4 + 1 part Assay Diluent	0.250
P6	Assay Diluent	0.000

- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water. Any remaining solution should be stored at 2-8⁰C.
- **Assay Diluent (1x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Any remaining solution should be stored at 2-8⁰C.

- **Plasminogen:** Add 1.2 ml reagent grade water. Any remaining solution should be frozen at -20°C .
- **Plasmin Substrate:** Add 0.55 ml of reagent grade water. Any remaining solution should be frozen at -20°C .

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 for specific sample binding and at 37°C for chromogenic activity assay. Seal the plate with sealing tape at each step.
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely and store in a vacuum desiccator to minimize exposure to water vapor.
- Add 200 μl of tPA Standard or Sample per well. Cover wells and incubate 3 hours in room temperature. Start the timer after the last sample addition.
- Wash five times with 200 μl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 μl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Freshly prepare the desired volume of the Assay Mix by combining the following reagents according to the number of wells in the assay (n) plus one.

<u>Reagents</u>	<u>n=1</u>
Assay Diluent	170 μl
Plasminogen	20 μl
Plasmin Substrate	10 μl

- Add 200 μl of the above Assay Mix to each well. Mix gently. Seal the plate with sealing tape and incubate overnight at 37°C
- Read the absorbance at 405 nm at 22 hours and continue reading every 1 hour through 26 hours.

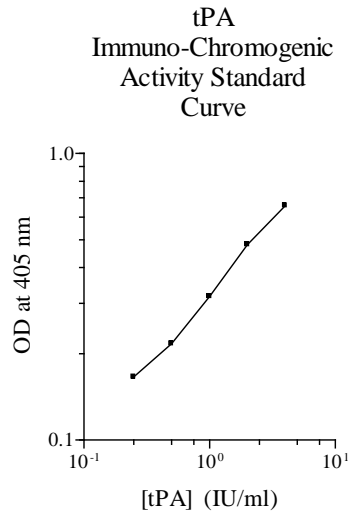
Standard or Sample	200 μl
<i>Incubate room temperature, 3 hours</i>	
<i>Wash</i>	
Assay Mix	200 μl
<i>Incubate at 37°C, overnight</i>	
Read the absorbance at 405 nm at zero minutes for background O.D.	
Read the absorbance at 405 at 22 hours and continue reading every 1 hour through 26 hours	

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

1. The minimum detectable dose of tPA is typically ~ 0.1 IU/ml.
2. This kit has no cross reactivity with uPA.
3. This assay recognizes both natural and recombinant human tPA.

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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