PLAT (Human) Chromogenic Activity Assay Kit

Catalog Number KA0974

96 assays

Version: 04

Intended for research use only

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Introduction

Background

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 the Assay

The PLAT (Human) 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, 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.

General Information

Materials Supplied

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

List of component

Component	Amount
Microplate: One 96-well polystyrene microplate (12 strips of 8 wells).	96 (8x12) wells
Sealing Tapes: Pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Assay Diluent	30 mL
tPA Standard: human tPA (32 IU).	1 vial
Human Plasminogen	1 vial
Plasmin Substrate	2 vials

Storage Instruction

- ✓ Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- ✓ Store Plasminogen, Standard, and Plasmin Substrate at -20°C.
- ✓ Store Microplate and Assay Diluent at 2-8°C. Unused microplate wells may be stored and resealed.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channel).
- ✓ Deionized or distilled reagent grade water.
- ✓ Incubator (37°C).

Precautions for Use

- ✓ 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.

Assay Protocol

Reagent Preparation

- ✓ Standard Curve: Reconstitute 32 IU of Human tPA Standard with 0.8 mL of Assay Diluent to generate a solution of 40 IU/mL. Allow the standard to sit for 15 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 solutions. Assay Diluent serves as the zero standard (0 µg/mL). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[tPA] (IU/mL)
P1	Standard (40 IU/mL)	40.00
P2	1 part P1 + 3 part Assay Diluent	10.00
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

- ✓ Plasminogen: Add 1.2 mL reagent grade water. Allow the plasminogen to sit for 15 minutes with gentle agitation prior to use; keep the vial on ice. Any remaining solution should be frozen at -20°C and used within 30 days.
- ✓ Plasmin Substrate: Add 0.55 mL reagent grade water. Allow the plasmin substrate to sit for 15 minutes with gentle agitation prior to use; keep the vial on ice. Any remaining solution should be frozen at -20°C and used within 30 days.

Sample Preparation

- ✓ 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 < -80°C. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as an antibcoagulant.)
- ✓ Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 15 minutes at 4°C to remove debris. Collect supernatants and assay. Samples can be store at < -80°C. Avoid repeated freeze-thaw cycles.

Assay Procedure

1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (25°C) for specific sample binding and at 37°C for chromogenic activity assay. Seal the plate with sealing tape at each step.
2. Remove excess microplate strips from the plate frame.
3. 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.

Assay Mix Reagents	N =1 well
Assay Diluent	60 µL
Plasminogen	10 µL
Plasmin Substrate	10 µL

4. Add 80 µL of the above Assay Mix to each well.
5. Add 20 µL of Human tPA Standard or samples per well and mix gently.
6. Read the absorbance at 405 nm at zero minutes for background O.D. Seal the plate with sealing tape. Incubate the plate at 37°C in a humid incubator to avoid drying the plate.
7. For HIGH tPA activity samples, read the absorbance at 405 nm every hour up to 8 hours.
8. 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°C, read the absorbance at 405 nm every hour for 8 hours.	
Low tPA Activity Samples: Incubate 37°C, read the absorbance at 405 nm every hour from 20 hours up to 26 hours.	

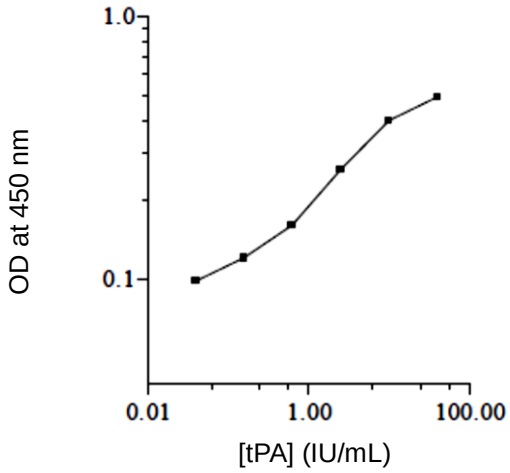
✓ Summary

1. Add 80 µL of Assay Mix and 20 µL of Standard/Sample. Incubate at 37°C for 1 hour.
2. Read at 405 nm eery hour for 8 hours. Read at 405 nm every hour from 20 hours up to 26 hours.

Data Analysis

Calculation of Results

- ✓ 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 (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.



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.

Resources

References

1. Vassalli, J.D. *et al.* (1991) *J. Clin. Invest*. 88: 1067
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3. Duffy, M.J. *et al.* (1992) *Fibrinolysis* 6: 55
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Plate Layout

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