

Tissue Plasminogen Activator (tPA) Activity Assay Kit (Colorimetric)

12/18

(Catalog # K178-100; 100 assays; Store at -20°C)

I. Introduction:

Tissue plasminogen Activator (tPA, PLAT or tissue-type plasminogen activator; EC 3.4.21.68) is a serine protease that is found in blood & some tissues. tPA is the major intravascular activator of plasminogen. Thus, tPA is a key enzyme and the primary initiator of fibrinolysis in the vascular system where it plays an important role in the breakdown of blood clots. tPA has therapeutic uses and it is known as the “clot buster” in clinical settings. High tPA activity in blood leads to hyperfibrinolysis and causes excessive bleeding; while low tPA activity leads to hypofibrinolysis, a condition that increases patient’s risks of suffering an arterial thrombosis. BioVision’s Tissue Plasminogen Activator (tPA) Activity Assay Kit utilizes the ability of active tPA to hydrolyze the synthetic substrate releasing pNA (chromophore). The released pNA can be easily quantified by OD at 405 nm. Our kit uses a unique combination of Substrate and Inhibitor Mix that specifically detects tPA in a variety of Biological Samples. Other enzymes with similar catalytic properties [i.e. uPA (urokinase or urokinase-type plasminogen activator)] do not interfere with the assay. Our assay kit is simple, specific and can detect as low as 5 µU of tPA activity.



II. Applications:

- Measurement of tPA activity in various Biological Fluids, Tissues and Cell Culture Supernatants.
- Analysis and study of Fibrinolytic system.

III. Sample Type:

- Biological Fluids: Plasma, Saliva, etc.
- Tissue Homogenates: Kidney.
- Cell Culture Supernatants: i.e. EA.hy926.

IV. Kit Contents:

Components	K178-100	Cap Code	Part Number
tPA Assay Buffer	50 ml	NM	K178-100-1
tPA Substrate	100 µl	Red	K178-100-2
tPA Positive Control	1 vial	Green	K178-100-3
Inhibitor Mix (10 mM)	20 µl	Blue	K178-100-4
pNA Standard (0.1 M)	20 µl	Yellow	K178-100-5

V. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer (ELISA reader)
- 96-well clear plate with flat bottom
- Dounce Tissue Homogenizer (Cat. #1998)
- 10K Spin Column (Cat. #1997)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protect from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Upon opening, use within two months.

- **tPA Assay Buffer:** Store at either 4 °C or -20 °C. Bring to room temperature before use.
- **tPA Substrate:** Store at -20°C. Protect from light. Bring to room temperature before use.
- **tPA Positive Control:** Reconstitute with 100 µl tPA Assay Buffer. Pipette up and down to mix well. Aliquot and store at -20°C. Keep on ice while in use.
- **Inhibitor Mix:** Store at -20 °C. Keep on ice while in use.
- **pNA Standard:** Store at -20 °C. Bring to room temperature before use.

VII. Tissue Plasminogen Activator Activity Assay Protocol:

1. Sample Preparation:

Plasma: Prepare Acidified Plasma* (for complete detail, see *Plasminogen Activator and Plasminogen Activator Inhibitor Activities in a Reference Population, Am J Clin Pathol, 89:747-52, 1988*):

- Mix Blood with 0.1 M citrate (anticoagulant) at a ratio of 9:1 and immediately centrifuge at room temperature for 10 min.
- Collect plasma and acidify sample immediately: mix equal volumes of Plasma and 1 M sodium acetate (pH 4.0; ratio 1:1). Mix the solution by inverting a couple of times and incubate mixture at room temperature for 15 min. Aliquot and store at -80 °C.

Prepare a 20-fold dilution of Acidified Plasma in tPA Assay Buffer. Prepare duplicate wells by adding 10 µl of Diluted Acidified Plasma into well(s) of a 96-well clear plate (labeled as “Sample” and “Sample Background Control”).

Tissue: Add 200–300 µl ice-cold tPA Assay Buffer per 100 mg of sample (wet weight). Homogenize using a Dounce homogenizer (BioVision: Cat. #1998) and keep on ice for 10 min. Centrifuge the samples at 12,000 x g for 10 min at 4 °C. Collect the supernatant.

Cell Culture Supernatants: Grow endothelial cells to confluency (usually 1-2 days). Collect the cell culture media and centrifuge at 3000 x g for 15 min at 4 °C to remove debris. Collect the supernatant.

Saliva: Collect Saliva Samples and centrifuge at 3000 x g for 15 min at 4 °C to remove debris. Collect the supernatant.

For Tissue, Cell Culture Supernatants and Saliva: Small molecules may interfere with the assay. We recommend sample ultrafiltration using 10K spin column (BioVision: Cat. #1997): Centrifuge samples (10000 x g, 4 °C, 10 min), discard filtrate and collect ultraconcentrate. Add fresh tPA Assay Buffer and bring back ultraconcentrate to its initial volume. Repeat this step 2-3 times and collect the ultraconcentrate to the initial volume. **For Samples and Sample Background Control:** Prepare duplicate wells by adding 2-20 µl of prepared samples into well(s) of a 96-well clear plate (labeled as “Sample” and “Sample Background Control”).

Positive Control, add 6-10 µl of reconstituted tPA into desired wells(s).

Adjust the volume of Positive Control, Sample wells, and Sample Background Control to **60 µl/well** with tPA Assay Buffer.

Note:

- a. Heparin treated plasma is not appropriate sample as Heparin can form complexes with t-PA.
- b. The tPA activity would be underestimated without acidifying the plasma due to the association of t-PA with Plasminogen Activator Inhibitor Type 1 (see Fig c)

2. Inhibitor Mix Preparation: Prepare a 10-fold dilution of Inhibitor Mix to 1 mM (i.e. Dilute 2 µl of Inhibitor Mix stock solution with 18 µl tPA Assay Buffer); further prepare a 200-fold dilution of inhibitor Mix to 5 µM (i.e. Dilute 2 µl of 1 mM Inhibitor Mix to 398 µl tPA Assay Buffer). Add 20 µl of Diluted Inhibitor Mix to each well containing Positive Control, Sample and Sample Background Control. *The volume of each well is 80 µl. Mix well and incubate the plate at 37 °C for 10 min, avoid light.*

3. Standard Curve Preparation: Prepare a 20-fold dilution of pNA Standard to 5 mM (i.e. Dilute 2 µl of 0.1 M pNA Standard into 38 µl tPA Assay Buffer). Add 0, 2, 4, 6, 8, 10 µl of 5 mM pNA Standard into each well individually. Adjust the volume to 100 µl/well with tPA Assay Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of pNA Standard.

4. Substrate Mix Preparation: Prepare a 20-fold dilution of tPA Substrate Stock solution (i.e. Dilute 5 µl of tPA stock Substrate with 95 µl tPA Assay Buffer), mix well. After 10 min incubation (see Step 2), add 20 µl of Diluted tPA substrate to each well containing the test samples and tPA positive Control. Add 20 µl of tPA Assay Buffer into well(s) containing Sample Background Control. *The total volume in every well (i.e. standards, samples, positive control, sample background controls) should be 100 µl.*

5. Measurement: Measure absorbance immediately at 405 nm in kinetic mode for 40-60 min at 37 °C. The pNA Standard(s) (see Step 3) can be read in End-point mode. Choose two time points (t₁ & t₂) in the linear range of the plot and obtain the corresponding values for the absorbance (OD₁ and OD₂).

6. Calculation: Subtract 0 Standard reading from all Standards readings. Plot the pNA Standard Curve. Calculate the signal from tPA of the test sample: $\Delta OD_S = OD_2 - OD_1$ and background signal from paired Sample Background Control ($\Delta OD_{BC} = OD_2 - OD_1$). Subtract the Sample Background Control reading from its paired Sample reading ($A = \Delta OD_S - \Delta OD_{BC}$). Apply **A** to the pNA Standard Curve to get **B** - nmol of pNA generated during the reaction time ($\Delta t = t_2 - t_1$).

$$\text{Sample tPA Activity} = \frac{(\text{B test sample} - \text{B Sample Background control})}{\Delta t \cdot V} * D = \text{nmol/min/ml} = \text{mU/ml}$$

Where: **B** = pNA amount from Standard Curve (nmol)

Δt = Reaction time (min)

V = Sample volume added into the reaction well (ml)

D = Dilution factor

Unit Definition: One unit of tPA activity is the amount of enzyme that releases 1.0 µmol of pNA per min at pH 8.8 at 37 °C.

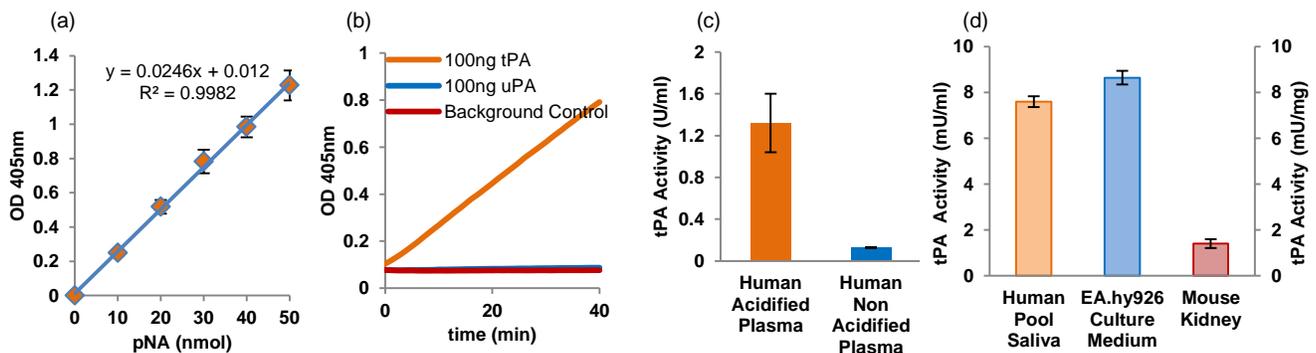


Figure: (a) pNA Standard Curve. (b) Detection of purified tPA (100 ng) and uPA (100 ng) activities - the kit with the combination of Substrate and Inhibitor Mix can effectively discriminate tPA activity from uPA activity. (c) Measurement of tPA activity in Human Pooled Acidified Plasma (10 µl, 1:20 diluted) and Human Pooled Non- acidified Plasma (10 µl, 1:20 diluted). (d) Measurement of tPA activity in Human Pooled Saliva (10 µl, ultraconcentrate), EA.hy926 Culture Supernatants (20 µl, ultraconcentrate) and Mouse Kidney Extracts (95 µg protein). All assays were performed following kit protocols.

VIII. RELATED PRODUCTS:

- Plasmin Activity Assay Kit (Fluorometric) (K381)
- Urokinase Inhibitor Screening Kit (Fluorometric) (K727)
- Factor XIa Activity Assay Kit (Colorimetric) (K973)
- Plasma Kallikrein Inhibitor Screening Kit (Colorimetric) (K989)

- Plasmin Inhibitor Screening Kit (Fluorometric) (K382)
- Urokinase Activity Fluorometric Assay Kit (K728)
- Factor XIIa Activity Assay Kit (Colorimetric) (K994)
- Plasma Kallikrein Activity Assay Kit (Colorimetric) (K997)

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