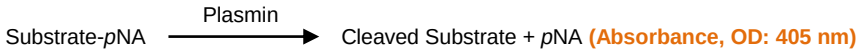
Plasmin Activity Assay Kit (Colorimetric)

06/19

(Catalog # K945-100; 100 assays; Store at -20°C)

I. Introduction:

Plasmin (EC 3.4.21.7) is a serine protease that is mainly present in plasma. It plays a key role in the degradation of fibrin clots in the blood and extracellular matrix protein components. Plasmin is involved in various physiological and pathological processes, including fibrinolysis, thrombolysis, wound healing and cancer progression. Malignant cells have been shown to enhance the generation of plasmin that can modify the tumor microenvironment and contribute to cell invasion. High concentrations of plasmin may lead to fibrinolysis and uncontrollable bleeding, while deficiency of plasmin may lead to thrombus that causes myocardial infarction and stroke. The proteolytic activity of plasmin is tightly regulated either by the activation of its precursor, plasminogen or its inhibition by the endogenous plasmin inhibitors such as plasminogen activator inhibitor-1 or α2-Antiplasmin. BioVision's Plasmin Activity Assay Kit utilizes the ability of active plasmin to hydrolyze the synthetic substrate thereby releasing *p*NA (chromophore), which can be easily quantified at OD 405 nm. The stable colorimetric signal is directly proportional to the plasmin activity in samples. The kit includes a specific inhibitor that can be used to compensate for the potential non-specific background in unknown samples. Our assay kit is simple, specific and can detect as low as 40 µU of plasmin activity in samples.



II. Applications:

- Measurement of Plasmin activity in Biological Samples: e.g. Plasma
- Analysis and study of Fibrinolytic system

III. Sample Type:

- Biological Fluids: Plasma, etc.

IV. Kit Contents:

Components	K945-100	Cap Code	Part Number
Plasmin Assay Buffer	50 ml	NM	K945-100-1
Plasmin Substrate	400 µl	Red	K945-100-2
*p*NA Standard	20 µl	Yellow	K945-100-3
Plasmin Positive Control	15 µl	Green	K945-100-4
Plasmin Inhibitor Mix	1 vial	Blue	K945-100-5

V. User Supplied Reagents and Equipment:

- Multi-well spectrophotometer
- 96-well clear plate with flat bottom
- dH_2O

VI. Storage Conditions and Reagent Preparation:

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

- **Plasmin Assay Buffer:** Ready to use. Store at either 4 ºC or -20 ºC. Bring to room temperature (RT) before use.
- **Plasmin Substrate:** Ready to use. Store at -20 °C. Protect from light. Keep on ice while in use.
- ***p*NA Standard (0.1 M in DMSO):** Store at -20 ºC. Bring to RT before use.
- **Plasmin Positive Control:** Store at -20 °C. Avoid repeated freeze-thaw cycles. Keep on ice while in use.
- **Plasmin Inhibitor Mix:** Reconstitute using 30 µl dH_2O. Pipette up and down to mix well. Store at -20 ºC. Keep on ice while in use.

  Note: Plasmin Inhibitor Mix must be prepared in dH_2O to preserve its activity.

VII. Plasmin Activity Assay Protocol:

1. Sample Preparation:

Plasma: Collect whole blood into commercially available EDTA-treated tubes. Cells are removed from Plasma by centrifugation for 10 min at 1,000-2,000 g using a refrigerated centrifuge. Collect the supernatant and centrifuge for another 15 min at 2,000 g to deplete the platelets in the Plasma Sample. Collect the supernatant and keep the Samples at 2-8 °C while handling. Prepare a 20-fold dilution of the EDTA-treated Plasma in Plasmin Assay Buffer. Add 10-50 µl of diluted Plasma into duplicate wells of a 96-well clear plate labeled as Sample and Sample Background Control.

Plasmin Positive Control: Prepare 100-fold dilution of the Plasmin Positive Control by adding 2 µl of Plasmin Positive Control into 198 µl Plasmin Assay Buffer. Add 4-10 µl of diluted Plasmin Positive Control into desired well(s). **Note:** Do not store the diluted Plasmin Positive Control.

Adjust the volume of **Positive Control**, **Sample(s)**, and **Sample Background Control** to **60 µl/well** with Plasmin Assay Buffer.

Notes:

a. If the EDTA-treated plasma is not analyzed immediately, it should be stored at -80 ºC or lower. It is important to avoid multiple freeze-thaw cycles.

b. Heparin-treated plasma is not recommended for the assay. This is because Heparin acts as an uncompetitive inhibitor of the plasmin substrate used in this kit.

c. Frozen/thawed Citrated-treated plasma generates Kallikrein activity which might affect the evaluation of plasmin activity.

2. **Standard Curve Preparation:** Prepare a 5 mM *p*NA Standard solution by diluting the stock 0.1 M *p*NA Standard (i.e. Dilute 2 µl of 0.1 M *p*NA Standard into 38 µl Plasmin Assay Buffer). Add 0, 2, 4, 6, 8, 10 µl of 5 mM (5 nmol/µl) *p*NA Standard into each well(s). Adjust the volume to 100 µl/well with Plasmin Assay Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of *p*NA Standard.
3. **Inhibitor Mix Preparation:** Prepare a 40-fold dilution of the Plasmin Inhibitor Mix (i.e. Dilute 2 µl of the Stock Plasmin Inhibitor Mix into 78 µl Plasmin Assay Buffer). Add 20 µl of the Diluted Plasmin Inhibitor Mix to the Sample Background Control well(s). Add 20 µl of Plasmin Assay Buffer to the Positive Control and Sample well(s). **Mix and incubate the plate at 37 ºC for 30 min**, avoid light.

   Note: Do not store the unused Diluted Inhibitor Mix. Prepare immediately before using.
4. **Reaction Mix Preparation:** Mix enough reagents for the number of assays to be performed. For each well, prepare 20 µl Reaction Mix containing:

	Reaction Mix
Plasmin Assay Buffer	16 µl
Plasmin Substrate	4 µl

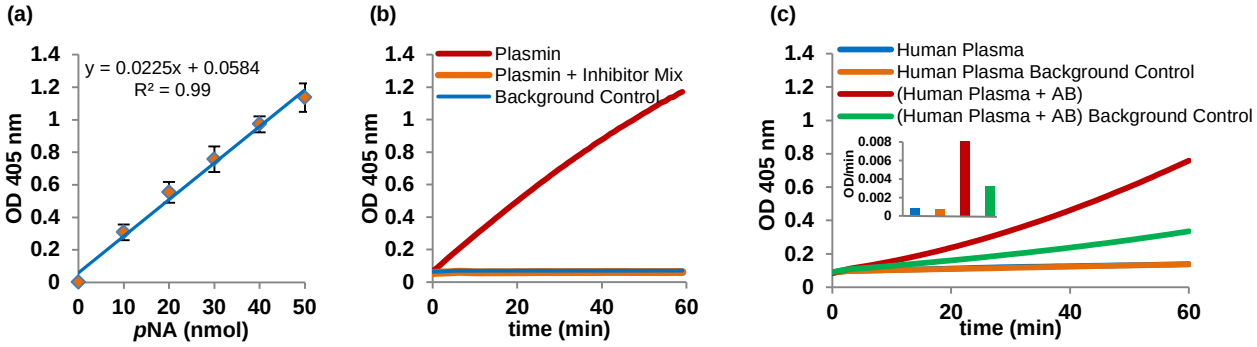
After 30 min incubation (see Step 3), add 20 µl Reaction Mix to Sample, Sample Background Control, and Positive Control wells. Mix well. The total volume of the Standard, Sample, Positive Control, Sample Background Control wells should be 100 µl/well.

5. **Measurement:** Measure absorbance immediately at 405 nm in a kinetic mode for 30-60 min at 37°C. The *p*NA Standard can be read in End-point mode. For Samples, choose any two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD_1 and OD_2).
6. **Calculation:** Subtract 0 Standard reading from all Standards readings. Plot the *p*NA Standard Curve. Calculate the signal from Plasmin in the Sample (ΔOD_S), ($\Delta OD_S = OD_2 - OD_1$) and the Background signal from the Sample Background Control (ΔOD_{BC}): ($\Delta OD_{BC} = OD_2 - OD_1$). Subtract the Sample Background Control reading from its paired Sample reading ($\Delta OD_S - \Delta OD_{BC}$) and apply to the *p*NA Standard Curve to get **B** nmol of *p*NA generated during the reaction time ($\Delta t = t_2 - t_1$).

$$\textbf{Sample Plasmin Activity} = \frac{(\text{B Sample} - \text{B Sample Background Control})}{\Delta t * V} * D = \text{nmol/min/ml} = \text{mU/ml}$$

Where: **B** = *p*NA amount from Standard Curve (nmol)
V = Sample volume added into the reaction well (ml)
Δ**t** = Reaction time (min)
D = Dilution factor (D=1, for undiluted Samples)

Unit Definition: One unit of Plasmin activity is the amount of enzyme that releases 1.0 µmol of *p*NA per min at pH 8.4 at 37ºC.



Figures: (a) *p*NA Standard Curve. (b) Measurement of purified Human Plasmin with or without the Plasmin Inhibitor Mix. (c) Measurement of Plasmin activity in Human Pooled EDTA-treated Plasma Sample (50 µl, 1:20 dilution) in the presence and absence of Activating Buffer containing Urokinase (BioVision #7696). All assays were performed following kit protocols.

VIII. Related Products:

Plasmin Activity Assay Kit (Fluorometric) (K381)
Plasmin Inhibitor Screening Kit (Fluorometric) (K382)
Plasmin, Human Plasma (4089)
Plasminogen, Human Plasma (7549)
Alpha 2 Antiplasmin, Human Plasma (7295)
Plasmin Sepharose Beads (7926)
Tissue Plasminogen Activator Activity Assay Kit (K178)
TPA, Human Recombinant (P1324)
Human Recombinant PAI-1 (6377)
Serpin E1/PAI-1, human recombinant (4731)
Urokinase, human recombinant (7696)
Pro-Urokinase, human recombinant (7695)
Plasma Kallikrein Inhibitor Screening Kit (Colorimetric) (K989)
Plasma Kallikrein Activity Assay Kit (Colorimetric) (K997)

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