# Plasmin Activity Assay Kit (Fluorometric)

*12/14*

**(Catalog # K381-100, 100 assays, Store kit at -20°C)**

## I. Introduction:

Plasmin (EC 3.4.21.7) is a serine protease occurring in plasma as plasminogen. Upon activation via cleavage by plasminogen activators; plasmin solubilizes fibrin clots and activates and/or degrades compounds of the coagulation and complement systems. Plasminogen-Plasmin system has also been implicated in a wide variety of physiologic and pathologic processes, including tumor growth, invasion and metastasis. BioVision's Plasmin activity assay kit utilizes the ability of Plasmin to proteolytically cleave a synthetic substrate and release a fluorophore, AMC, which can be easily quantified by fluorescence microplate readers. This assay kit is simple, rapid and can detect Plasmin activity as low as 10 ng in a variety of samples.

Plasmin Substrate-AMC $\xrightarrow{\text{Plasmin}}$ Cleaved substrate + AMC (Fluorescence)

## II. Applications:

- Determine activity of pure Plasmin
- Detect the activity of Plasmin in plasma

## III. Kit Contents:

| Components | K381-100 | Cap Code | Part Number |
|---|---|---|---|
| Plasmin Assay Buffer | 15 ml | WM | K381-100-1 |
| Plasmin Dilution Buffer | 1.5 ml | Clear | K381-100-2 |
| Plasmin Enzyme Standard (1 mg/ml) | 5 µl | Green | K381-100-3 |
| Plasmin Substrate | 0.2 ml | Red | K381-100-4 |

## IV. User Supplied Reagents and Equipment:

- 96-well white microplate with flat bottom.
- Multi-well spectrophotometer.

## V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read entire protocol before performing the assay.

- **Plasmin Assay Buffer:** Bring to room temperature before use.
- **Plasmin Enzyme Standard:** Aliquot and store at -80°C. Avoid repeated freeze/thaw.

## VI. Plasmin Activity Assay Protocol:

1. **Sample Preparation:** Add 2-50 µl of sample containing Plasmin per well of 96-well plate and adjust the volume to 50 µl with Plasmin Assay Buffer.

   **Note:** (Optional) for samples having fluorescence background, prepare in parallel sample background control well(s) containing sample only and adjust the volume to 100 µl/well with Plasmin Assay Buffer.

2. **Standard Curve Preparation**: Prepare working solution of 10 ng/µl Plasmin Enzyme by adding 198 µl of Plasmin Dilution Buffer to 2 µl of Plasmin Enzyme Standard. Mix well by pipetting up and down. Add 0, 5, 10, 15, 20, and 25 µl of Plasmin Enzyme working solution (10 ng/µl) into a series of wells in a 96-well plate to prepare 50, 100, 150, 200, and 250 ng/well of Plasmin Enzyme Standard. Adjust the volume to 50 µl/well with Plasmin Assay Buffer.

   **Note**: The unused Plasmin Enzyme working solution may be stored at -20°C for two weeks or -80°C for up to 2 months.

3. **Plasmin Substrate Mix:** Prepare enough reagents for the number of assays to be performed. Prepare 50 µl of Substrate Mix for Standard and sample wells.

   | | |
   |---|---|
   | Plasmin Assay Buffer | 48 µl |
   | Plasmin Substrate | 2 µl |

   Mix and add 50 µl of Plasmin Substrate Mix into Standard and sample well(s). Mix well.

4. **Measurement:** Measure fluorescence in kinetic mode for 10-20 min. at 37°C (Ex/Em = 360/450 nm). Choose two time points ($T_1$ and $T_2$) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU1 and RFU2).

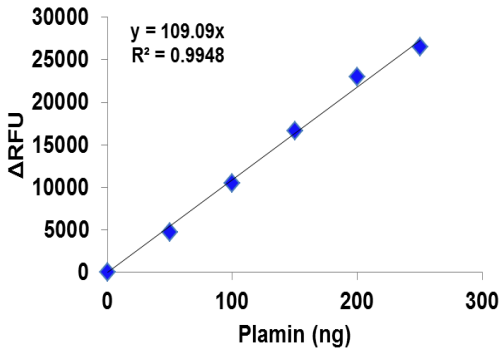
5. **Calculations**: Subtract 0 Standard reading from all readings. Plot the Plasmin Standard Curve. Apply sample's ΔRFU to Plasmin Standard Curve to obtain corresponding Plasmin (B, in ng) and calculate the activity of Plasmin in the sample as:

$$\textbf{Sample Plasmin Activity} = \frac{B}{V} \times \textit{Dilution Factor} = \frac{ng}{ml} = \frac{\mu g}{L}$$

Where B is Plasmin amount from Standard Curve (ng)
V is the sample volume added into the reaction well (ml)

**Note:** If the sample background control reading is significant, subtract the sample background control reading from sample reading.

(a)




(b)

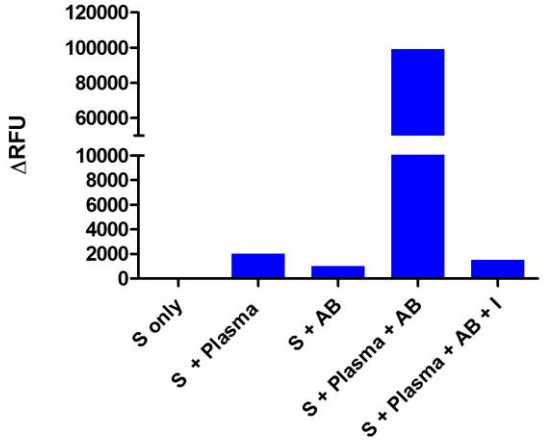



**Figure**: (a) Standard plot of Plasmin activity. (b) Plasmin activity was measured in plasma samples in the presence and absence of a Plasmin inhibitor, Aprotinin. S = Substrate, I = Inhibitor, AB = Activation Buffer containing Urokinase (Cat. # 7696). Assays were performed following the kit protocol.

## VII. RELATED PRODUCTS:

Plasmin, Human Plasma (4089)
AntiPlasmin III (7298)
Urokinase, human recombinant (7696)
Urokinase Sepharose Beads (7927)
Fibrinogen (plasminogen depleted), Human Plasma (7692)
Angiostatin K1-3, human recombinant (4920)
PAI-1 Antibody (5579)
Serpin E1/PAI-1, human recombinant (4731)
Plasminogen, Human Plasma (7549)
Plasmin Sepharose Beads (7926)
Pro-Urokinase, human recombinant (7695)
Urokinase Activity Fluorometric Assay Kit (K728)
Angiostatin Human (4919)
Human Recombinant PAI-1 (6377)
uPAR Antibody (3440)
Alpha 2 Antiplasmin, Human Plasma (7295)

***FOR RESEARCH USE ONLY! Not to be used on humans.***