



# 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

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<sub>1</sub> and T<sub>2</sub>) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU1 and RFU2).
- Calculations: Subtract 0 Standard reading from all readings. Plot the Plasmin Standard Curve. Apply sample's ∆RFU to Plasmin Standard Curve to obtain corresponding Plasmin (B, in ng) and calculate the activity of Plasmin in the sample as:

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

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.



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**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)

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