

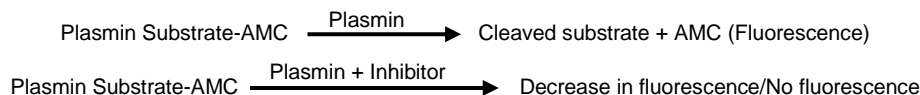
Plasmin Inhibitor Screening Kit (Fluorometric)

12/14

(Catalog # K382-100; 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. Plasmin inhibitors are critical in the treatment of hyperfibrinolysis-associated blood loss and related complications. Biovision's Plasmin Inhibitor Screening Kit utilizes the ability of Plasmin to cleave a synthetic AMC-based peptide substrate to release AMC, which can be detected by measuring its fluorescence at Ex/Em = 360/450 nm. In the presence of Plasmin specific inhibitors, the extent of cleavage reaction is reduced or completely abolished. The loss in the fluorescence intensity can be correlated to the amount of inhibitor present in the assay solution. The kit provides a simple and rapid method to screen potential inhibitors of Plasmin.



II. Applications:

- Screening potential inhibitors of Plasmin
- Characterizing/studying Plasmin inhibitors in plasma samples

III. Kit Contents:

Components	K382-100	Cap Code	Part Number
Plasmin Assay Buffer	15 ml	WM	K382-100-1
Plasmin Dilution Buffer	1.5 ml	Clear	K382-100-2
Plasmin Enzyme	15 µl	Green	K382-100-3
Plasmin Substrate	0.2 ml	Red	K382-100-4
Plasmin Inhibitor (Aprotinin, 0.6 mM)	0.1 ml	Blue	K382-100-5

IV. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plate is preferred for this assay.
- Multi-well spectrophotometer

V. Storage Condition 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:** Aliquot the stock solution and store at -80°C. Avoid repeated freeze/thaw.

VI. Plasmin Inhibitor Screening Protocol:

1. **Enzyme Solution Preparation:** Dilute Plasmin Enzyme 1:100 with Plasmin Dilution Buffer. Make as per the assay requirement. Mix well by pipetting up and down. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl of Plasmin enzyme solution.

Plasmin Assay Buffer	35 µl
Diluted Plasmin Enzyme	15 µl

Mix and add 50 µl of Plasmin Enzyme Solution into desired wells.

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

2. **Screening compounds, Inhibitor Control and Enzyme Control Preparations:** Dissolve candidate inhibitors into proper solvent. Dilute to 10X the desired test concentration with Plasmin Assay Buffer. Add 10 µl diluted test inhibitors (I) or Plasmin Assay Buffer (Enzyme Control, EC) into Plasmin Enzyme containing wells. As an Inhibitor Control (IC), add 1 µl Plasmin Inhibitor and 9 µl Plasmin Assay Buffer to Plasmin Enzyme well(s). Incubate at room temperature for 10-15 min.

3. **Plasmin Substrate Preparation:** For each well, prepare 40 µl of substrate solution.

Plasmin Assay Buffer	38 µl
Plasmin Substrate	2 µl

Mix and add 40 µl of Plasmin Substrate solution into each well. Mix well.

4. **Measurement:** Measure fluorescence in a kinetic mode for 10-20 min at 37°C (Ex/Em = 360/450 nm). Choose two time points (T₁ and T₂) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU₁ and RFU₂).
5. **Calculations:** Calculate the slope for all Samples (S), including Enzyme Control (EC), by dividing the net ΔRFU (RFU₂-RFU₁) values with the time ΔT (T₂-T₁).

$$\% \text{ Relative Inhibition} = \frac{\text{Slope of EC} - \text{Slope of S}}{\text{Slope of EC}} \times 100$$

Note: Irreversible inhibitors that inhibit the Plasmin activity completely at the tested concentration will have $\Delta\text{RFU} = 0$ and will show 100% Relative Inhibition.

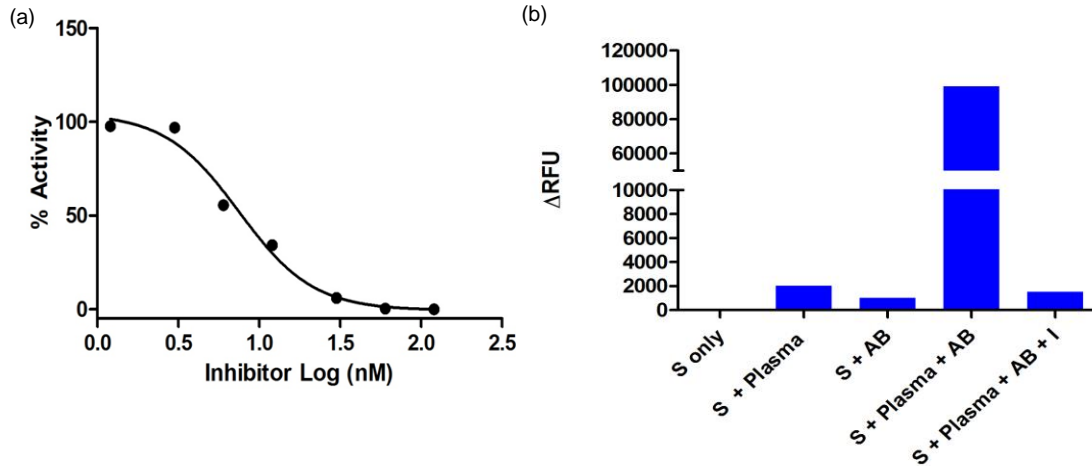


Figure: (a) Inhibition of Plasmin activity by a Plasmin Inhibitor (Aprotinin). (b) Plasmin activity was measured in plasma samples in the presence and absence of a Plasmin inhibitor, Aprotinin. S = Substrate, I = Inhibitor, AB = Activation Buffer containing Urokinase (Catalog # 7696). Assays were performed 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.