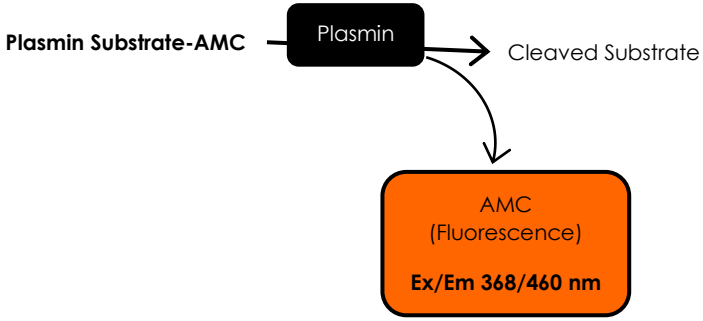
ab324630 – Plasmin Activity Assay Kit (Fluorometric)

For rapid, sensitive and accurate measurement of Plasmin activity in biological samples.
For research use only - not intended for diagnostic use.

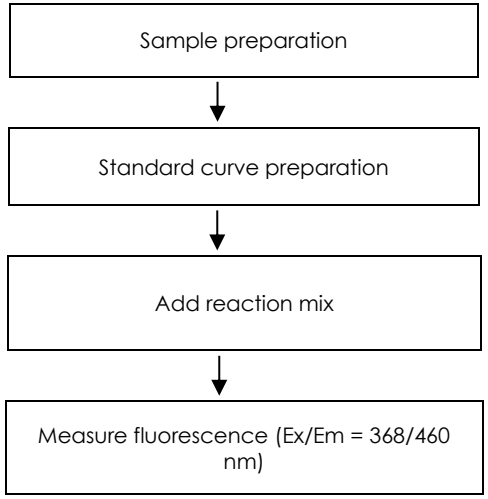
Background:

Plasmin (EC 3.4.21.7) is serine protease that is mainly present in plasma. It is secreted from the liver as a pro-enzyme (zymogen) called plasminogen that is cleaved to the active enzyme by a number of different enzymes in the fibrinolysis pathway, particularly tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). 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 tumour microenvironment and contribute to metastatic tumour invasion. High concentrations of plasmin may lead to fibrinolysis and uncontrollable bleeding, while deficit 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 (PAI-1) or a2-Antiplasmin. Abcam's Plasmin Activity Assay Kit utilizes the ability of plasmin to proteolytically cleave a synthetic peptide substrate and release AMC (fluorophore), which can be easily quantified by fluorescence at Ex/Em = 368/460 nm. The fluorometric signal is directly proportional to the plasmin activity in samples. Our assay kit can detect as low as 8.66 µU of plasmin activity in biological samples.



Assay Summary:

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.



QUICK ASSAY PROCEDURE

- Thaw kit components and aliquot components susceptible to freeze/thaws; get equipment ready.
- Prepare standard curve and positive control reactions.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard, positive control, and samples (50 µL).
- Prepare Plasmin Reaction Mix.
- Add 50 µL of Plasmin Reaction Mix to each well.
- Measure fluorescence (Ex/Em= 368/460 nm) in kinetic mode, or after a fixed 30-minute incubation at 37°C (protected from light) if performing an endpoint assay).

Precautions & Limitations:

Please read these instructions carefully prior to beginning the assay.
All kit components have been formulated and quality tested to function successfully as a kit.

- Modifications to the kit components or procedures may result in loss of performance.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

Storage and Stability:

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Reconstituted components are stable for 2 months. Do not use kit or components if they have exceeded the expiry date.

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
Plasmin Assay Buffer	15 mL	-20 °C	4 °C
Plasmin Positive Control	15 µL	-20 °C	-20 °C
Plasmin Dilution Buffer	1.5 mL	-20 °C	-20 °C
Plasmin Substrate	400 µL	-20 °C	-20 °C
AMC Standard (1 mM)	100 µL	-20 °C	-20 °C

Materials Required, Not Supplied:

These materials are not included in the kit, but will be required to successfully utilize this assay:
- Microplate reader capable of measuring fluorescence at Ex/Em = 368/460 nm
- 96 well black plate with flat bottom
- Centrifuge with refrigeration
- MilliQ water or other type of double distilled/deionized water (ddH_2O)

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

Plasmin Assay Buffer and **Plasmin Dilution Buffer** are ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

Plasmin Positive Control: Ready to use as supplied. Keep on ice while in use. Avoid repeated freeze/thaws and store at -20°C.

Plasmin Substrate: Ready to use as supplied. Keep on ice while in use. Avoid repeated freeze/thaws and protect from light. Store at -20°C.

AMC Standard: Provided as 1 mM stock solution. Store at -20°C, stable for at least 3 freeze/thaw cycles.

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.
- If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.

Prepare serial dilution of AMC Standard as follows:

1. Dilute 1 mM AMC Standard to 100 µM by adding 10 µL of 1 mM AMC Standard Stock to 90 µL of ddH_2O.
2. Add 0, 2, 4, 6, 8, and 10 µL of 100 µM AMC Standard into a series of wells in a 96 well black plate.
3. Adjust the volume to 50 µL/well with Plasmin Assay Buffer to generate 0, 200, 400, 600, 800, and 1000 pmol/well of AMC Standard.

ΔNOTE: To improve accuracy and reduce CVs, the standard curve may be prepared in duplicate, in a microplate or microcentrifuge tubes using the table below. Each standard mix has enough volume to set up duplicate readings (2 x 50 µL).

Standard #	Volume of 100 µM Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	AMC in well (pmole)
1	0	150	50	0
2	6	144	50	200
3	12	138	50	400
4	18	132	50	600
5	24	126	50	800
6	30	120	50	1000

Positive Control Preparation:

- Always prepare a fresh dilution of positive control for every use and discard working dilution after use.

1. Prepare a 100-fold dilution of Plasmin Positive Control by adding 2 µL of Plasmin Positive Control to 198 µL of Plasmin Dilution Buffer and mix well.
2. Add 25 µL of diluted Plasmin Positive Control to 96 well black plate and adjust the volume to 50 µL/well with Plasmin Assay Buffer.

Sample Preparation:

- We recommend testing several different doses of your sample to ensure the readings are within the linear range for the assay.

1. We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, flash freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples, and the readings can be lower than expected.

Plasma Samples:

1. Collect whole blood into EDTA-treated tubes.
2. Cells are removed from Plasma by centrifugation for 10 min at 1,000-2,000 g using a refrigerated centrifuge.
3. Collect the supernatant and centrifuge for another 15 mins at 2,000 g to deplete the platelets in the Plasma Sample.

4. Collect the supernatant and keep the Samples at 2-8° C while handling.
5. Prepare a 20-fold dilution of the EDTA-treated Plasma in Plasmin Assay Buffer.
6. Add 2-50 µL of diluted Plasma into duplicate wells of a 96-well black plate labeled as Sample and Sample Background Control.

ΔNOTE: 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. Freeze/thawed Citrated-treated plasma generates Kallikrein activity which may affect the evaluation of plasmin activity.

Assay Procedure:

- Keep enzymes and heat labile components and samples on ice during the assay.
- Equilibrate all other materials and prepared reagents to room temperature prior to use.
- We recommend pre-setting your microplate reader temperature to 37°C prior to performing the assay.
- We recommend that you assay all standards, controls, and samples in duplicate (or triplicate for enhanced precision in case of outliers).

1. Set up Reaction wells:
   - Standard wells = add 50 µL standard dilutions.
   - Sample wells = add 2 – 50 µL of diluted Plasma into duplicate wells and adjust the volume to 50 µL/well with Plasmin Assay Buffer.
2. Each well (standards, samples, and controls) requires 50 µL of Reaction Mix. To ensure consistency, use the table below to prepare a bulk solution of the Reaction Mix:

Component	Volume per well (µL)
Plasmin Assay Buffer	46
Plasmin Substrate	4

3. Mix bulk Reaction Mix by inversion. Add 50 µL of the bulk Reaction Mix to each well. Use a clean tip for each well. We recommend preconfiguring the fluorescence microplate reader settings and use of a multichannel pipette with a reagent reservoir to minimize lag time among wells.
4. Measure fluorescence (Ex/Em = 368/460 nm) in kinetic mode on a microplate reader at 37°C for 30 minutes.

ΔNOTE: Measuring fluorescence in kinetic mode is recommended for this assay to ensure that the measurements recorded are within the linear range of the reaction. Alternatively, the assay may be performed in endpoint mode by incubating the plate for 30 minutes at 37°C, protected from light, then immediately measuring fluorescence output (Ex/Em = 368/460 nm).

Calculations:

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean fluorescence value of the blank (Standard #1) from all standard and sample readings. This is the corrected fluorescence.
3. Plot the corrected fluorescence values for each standard as a function of the final concentration of AMC Standard (in pmoles/well).
4. Using a linear regression function, calculate the best fit curve through these points to construct the standard curve and determine the slope based on your standard curve data.
5. Extrapolate sample readings from the standard curve plotted using the following equation to determine the amount substrate metabolized in pmoles (*B*):

$$B = \left(\frac{Corrected\ fluorescence - (y - intercept)}{Slope}\right)$$

6. Concentration of Plasmin in the test samples is calculated as:

$$\textbf{Sample Plasmin Activity} = \frac{B}{\Delta T \times V} \times D = \textbf{pmoles/min/ml = µU/ml}$$

Where:

B is the amount of AMC produced, calculated from the standard curve (in pmoles)
ΔT is the linear phase reaction time $t_2 - t_1$ (in minutes, ΔT = 30 for an endpoint assay)
V is the amount of sample added to the well (in ml of fluid)
D is the sample dilution factor (if applicable, *D*=1 for undiluted samples)

Technical Hints

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

Technical Support

For technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)