ab204728

Plasmin Activity Assay Kit (Fluorometric)

Instructions for Use

For rapid, sensitive and accurate detection of Plasmin activity.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 2a, Last Updated 16 August 2023

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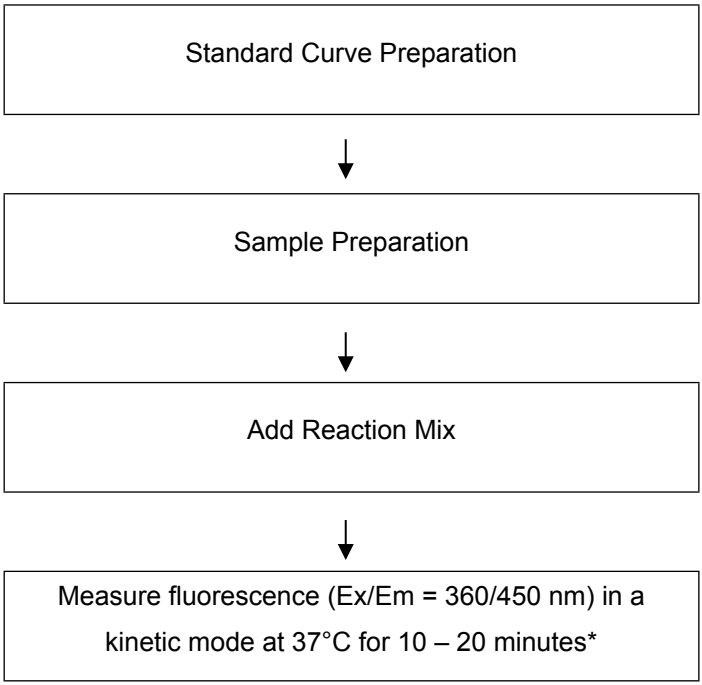
1. BACKGROUND

Plasmin Activity Assay Kit (Fluorometric) (ab204728) is based on the ability of Plasmin to proteolytically cleave a synthetic plasmin substrate and release a fluorophore, AMC, which can be easily quantified by fluorescence microplate readers at Ex/Em = 360/450 nm. This assay kit is simple, rapid and can detect Plasmin activity as low as 10 ng in a variety of samples.

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.

2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Plasmin Assay Buffer	15 mL	-20°C	-20°C
Plasmin Dilution Buffer	1.5 mL	-20°C	-20°C
Plasmin Enzyme/Plasmin Enzyme Standard	5 µL	-20°C	-80°C
Plasmin Substrate I/Plasmin Substrate	200 µL	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter Ex/Em = 360/450 nm
- 96 well plate with clear flat bottom preferably white
- Heat block or water bath

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Plasmin Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **Plasmin Dilution Buffer:**

Ready to use as supplied. Store at -20°C.

9.3 **Plasmin Enzyme/Plasmin Enzyme Standard:**

Ready to use as supplied. Store at -20°C. Avoid repeated freeze/thaw. ***NOTE:*** *once standard has been diluted (Step 10.1), store at -80°C and use within two months.*

9.4 **Plasmin Substrate I/Plasmin Substrate:**

Ready to use as supplied. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution (10 ng/µL) can be stored at -20°C for two weeks or -80°C for up to 2 months for later use.

10.1 Prepare 500 µL of 10 ng/µL Plasmin Enzyme/Plasmin Enzyme Standard by diluting 5 µL of the provided Plasmin Enzyme stock solution (1 mg/mL) with 495 µL of Plasmin Dilution Buffer.

10.2 Using 10 ng/µL Plasmin Enzyme/Plasmin Enzyme Standard, prepare standard curve dilution as described in the table in a microplate.

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End Conc Plasmin in well (ng/well)
1	0	150	50	0
2	15	135	50	50
3	30	120	50	100
4	45	105	50	150
5	60	90	50	200
6	75	75	50	250

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- 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, if that is not possible, we suggest that you snap freeze and store the samples immediately at -80°C upon extraction. 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.

11.1 Plasma:

Plasma samples can be tested directly by adding sample to the microplate wells.

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample.

11.2 Purified protein:

Purified protein can be used directly.

To find optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
- **Prepare all reagents, working standards, and samples as directed in the previous sections.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Plasmin Assay Buffer).
- Background control sample wells = 2 - 50 µL samples (adjust volume to 100 µL/well Plasmin Assay Buffer). ***NOTE:*** *for samples having fluorescence background.*

12.2 **Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction:

Component	Reaction Mix (µL)
Plasmin Assay Buffer	**48**
Plasmin Substrate I/Plasmin Substrate	**2**

Mix enough reagents for the number of assays (samples and standards) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions +1).

12.3 Add 50 µL of Reaction Mix into each standard and sample well. Do not add Reaction Mix to background control samples. Mix well.

12.4 Measure output on a fluorescent microplate reader at Ex/Em = 360/450 nm in a kinetic mode, every 2 – 3 minutes, for 10 – 20 minutes at 37°C protected from light.

NOTE: *Sample incubation time can vary depending on Plasmin (Plasmin) activity in the samples. We recommend measuring fluorescence in kinetic mode and then choosing two time points (T_1 and T_2) during the linear range.*

RFU value at T_2 should not exceed the highest RFU in the standard curve. For standard curve, do not subtract RFU_1 from RFU_2 reading.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean RFU value of the blank (Standard #1) from all standard and sample readings. This is the corrected RFU.

13.3 Plot the corrected RFU values for each standard as a function of the final concentration of plasmin.

13.4 Draw the best smooth curve through these points to construct the standard curve. Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5 Amount of Plasmin is calculated as:

$$\mathbf{\Delta RFU_{360/450nm} = (RFU_2 - RFU_{2BG}) - (RFU_1 - RFU_{1BG})}$$

Where:

RFU_1 is the sample reading at time T_1.

RFU_{1BG} is the background control sample at time T_1.

RFU_2 is the sample reading at time T_2.

RFU_{2BG} is the background control sample at time T_2.

13.6 Use the $\Delta RFU_{360/450nm}$ to obtain B (in ng) of Plasmin.

13.7 Activity of Plasmin in the test is calculated as:

$$Plasmin\ Activity = \left(\frac{B}{V}\right) * D = ng/mL = \ \mu g/L$$

Where:

B = Amount of Plasmin from Standard Curve (ng).

V = Original sample volume added into the reaction well (mL).

D = Sample dilution factor

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

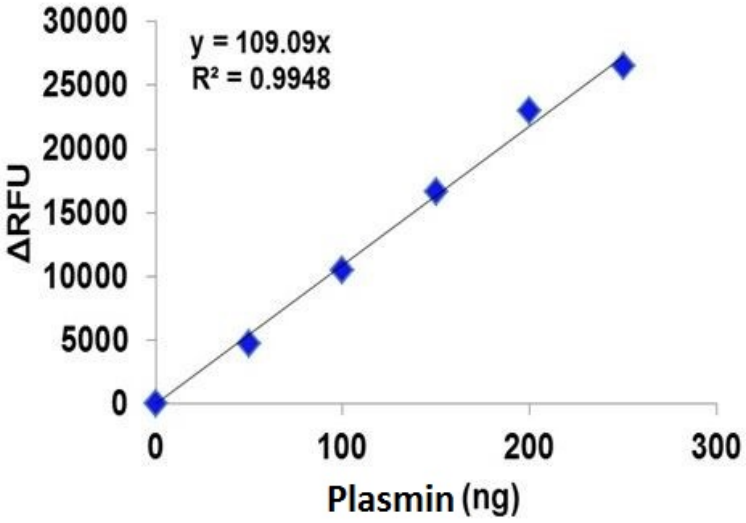


Figure 1. Typical Plasmin Standard calibration curve using fluorometric reading.

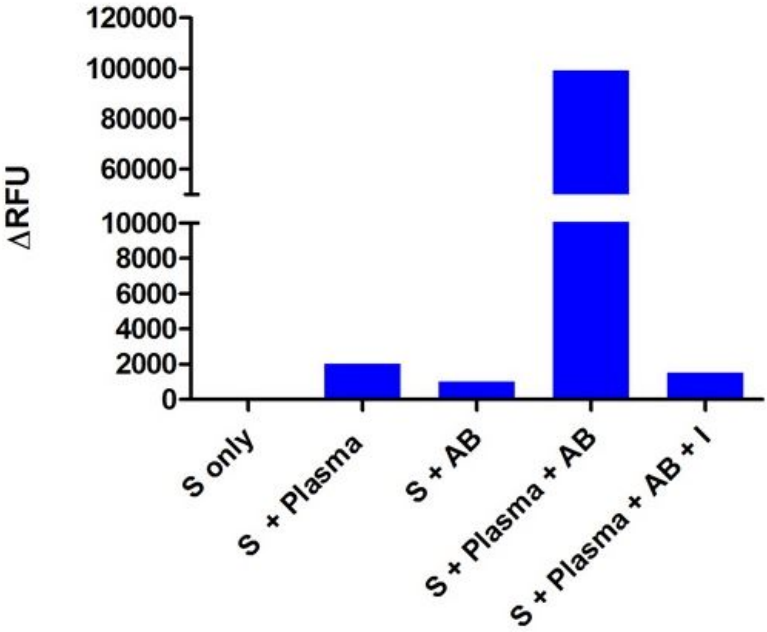


Figure 2. Plasmin activity was measured in plasma samples in the presence and absence of a Plasmin inhibitor, Aprotinin. S = Substrate, I = Inhibitor, AB = Activation Buffer containing Urokinase.

15. QUICK ASSAY PROCEDURE

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

- Prepare standard and prepare enzyme mix; get equipment ready.
- Prepare appropriate standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL) and samples (50 µL) and background control (100 µL) (optional) wells.
- Prepare Plasmin Reaction Mix (Number samples + standards + 1).

Component	Reaction Mix (µL)
Plasmin Assay Buffer	**48**
Plasmin Substrate I/Plasmin Substrate	**2**

- Add 50 µL of Plasmin Reaction Mix to the standard and sample wells.
- Incubate plate at 37°C during 10-20 minutes and read fluorescence at Ex/Em= 350/450 nm in a kinetic mode.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17.FAQ

Which chemicals or biological materials cause interference in this assay?

RIPA buffer – contains SDS which can denature proteins and affect enzyme activity.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)