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.
# Table of Contents

**INTRODUCTION**
1. BACKGROUND 2
2. ASSAY SUMMARY 3

**GENERAL INFORMATION**
3. PRECAUTIONS 4
4. STORAGE AND STABILITY 4
5. LIMITATIONS 4
6. MATERIALS SUPPLIED 5
7. MATERIALS REQUIRED, NOT SupPLIED 5
8. TECHNICAL HINTS 6

**ASSAY PREPARATION**
9. REAGENT PREPARATION 7
10. STANDARD PREPARATION 8
11. SAMPLE PREPARATION 9

**ASSAY PROCEDURE and DETECTION**
12. ASSAY PROCEDURE and DETECTION 10

**DATA ANALYSIS**
13. CALCULATIONS 12
14. TYPICAL DATA 14

**RESOURCES**
15. QUICK ASSAY PROCEDURE 15
16. TROUBLESHOOTING 16
17. FAQ 18
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.

\[
\text{Plasmin} \\
\text{Plasmin Substrate-AMC} \quad \rightarrow \quad \text{Cleaved substrate} \\
+ \text{AMC (Fluorescence)}
\]

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

Standard Curve Preparation

↓

Sample Preparation

↓

Add Reaction Mix

↓

Measure fluorescence (Ex/Em = 360/450 nm) in a kinetic mode at 37°C for 10 – 20 minutes*

*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 -20°C 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

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
<th>Storage Condition (After Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmin Assay Buffer</td>
<td>15 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Plasmin Dilution Buffer</td>
<td>1.5 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Plasmin Enzyme Standard</td>
<td>5 µL</td>
<td>-20°C</td>
<td>-80°C</td>
</tr>
<tr>
<td>Plasmin Substrate</td>
<td>200 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

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 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:**

  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 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 Standard, prepare standard curve dilution as described in the table in a microplate.

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

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:

<table>
<thead>
<tr>
<th>Component</th>
<th>Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmin Assay Buffer</td>
<td>48</td>
</tr>
<tr>
<td>Plasmin Substrate</td>
<td>2</td>
</tr>
</tbody>
</table>

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 absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance 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:

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

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.

![Typical Plasmin Standard calibration curve using fluorometric reading.](image1)

\[ y = 109.09x \]
\[ R^2 = 0.9948 \]

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

![Plasmin activity measured in plasma samples in the presence and absence of a Plasmin inhibitor, Aprotinin.](image2)

**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).

<table>
<thead>
<tr>
<th>Component</th>
<th>Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmin Assay Buffer</td>
<td>48</td>
</tr>
<tr>
<td>Plasmin Substrate</td>
<td>2</td>
</tr>
</tbody>
</table>

- 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.
# Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Use of ice-cold buffer</td>
<td>Buffers must be at room temperature</td>
</tr>
<tr>
<td></td>
<td>Plate read at incorrect wavelength</td>
<td>Check the wavelength and filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>Use of a different 96-well plate</td>
<td>Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate</td>
</tr>
<tr>
<td>Sample with erratic readings</td>
<td>Samples not deproteinized (if indicated on protocol)</td>
<td>Use PCA precipitation protocol for deproteinization</td>
</tr>
<tr>
<td></td>
<td>Cells/tissue samples not homogenized completely</td>
<td>Use Dounce homogenizer, increase number of strokes</td>
</tr>
<tr>
<td></td>
<td>Samples used after multiple free/thaw cycles</td>
<td>Aliquot and freeze samples if needed to use multiple times</td>
</tr>
<tr>
<td></td>
<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use</td>
</tr>
<tr>
<td></td>
<td>Presence of interfering substance in the sample</td>
<td>Check protocol for interfering substances; deproteinize samples</td>
</tr>
<tr>
<td>Lower/HIGHER readings in samples and standards</td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
</tr>
<tr>
<td></td>
<td>Allowing reagents to sit for extended times on ice</td>
<td>Always thaw and prepare fresh reaction mix before use</td>
</tr>
<tr>
<td></td>
<td>Incorrect incubation times or temperatures</td>
<td>Verify correct incubation times and temperatures in protocol</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>-------------------------------------------------------</td>
</tr>
<tr>
<td>Standard readings do not follow a linear</td>
<td>Pipetting errors in standard or reaction</td>
<td>Avoid pipetting small volumes (&lt; 5 µL) and prepare</td>
</tr>
<tr>
<td>pattern</td>
<td>mix</td>
<td>a master mix whenever possible</td>
</tr>
<tr>
<td></td>
<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the tubes</td>
</tr>
<tr>
<td></td>
<td>Standard stock is at incorrect concentration</td>
<td>Always refer to dilutions on protocol</td>
</tr>
<tr>
<td>Unanticipated results</td>
<td>Measured at incorrect wavelength</td>
<td>Check equipment and filter setting</td>
</tr>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>Troubleshoot if it interferes with the kit</td>
</tr>
<tr>
<td></td>
<td>Sample readings above/ below the linear range</td>
<td>Concentrate/ Dilute sample so it is within the linear range</td>
</tr>
</tbody>
</table>
17. FAQ

Which chemicals or biological materials cause interference in this assay?

RIPA buffer – contains SDS which can denature proteins and affect enzyme activity.
UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 108008523689 (中國聯通)

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp

Copyright © 2015 Abcam, All Rights Reserved. The Abcam logo is a registered trademark.
All information / detail is correct at time of going to print.