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ab273301 Plasmin Activity Assay Kit (Colorimetric)

View Kit datasheet: https://www.abcam.com/ab273301
(use https://www.abcam.cn/ab273301 for china, or
https://www.abcam.co.jp/ab273301 for Japan)

For the determination of plasmin activity in biologic fluids.

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.

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1. Overview

Plasmin Activity Assay Kit (Colorimetric) (ab273301) utilizes the ability of active plasmin to hydrolyze the synthetic substrate thereby releasing *p*NA (chromophore), which can be easily quantified at OD 405 nm. The stable colorimetric signal is directly proportional to the plasmin activity in samples. The kit includes a specific inhibitor that can be used to compensate for the potential non-specific background in unknown samples. Our assay kit is simple, specific and can detect as low as 40 µU of plasmin activity in samples.

2. Protocol Summary

Prepare Samples and Plasmin Positive Control as directed

Prepare all reagents as directed

Prepare standard curve and measure at OD = 405 nm.
Calculate slope.

Add Positive Control, Samples and Sample Background Control to appropriate wells and adjust volume to 60 µL

Add Diluted Plasmin Inhibitor Mix (20 µl) to Sample Background Control wells

Add Assay Buffer A1/Plasmin Assay Buffer (20 µl) to Positive Control and Sample wells

Incubate the plate at 37 °C for 30 min, avoid light.

Add Reaction Mix (20 µL)

Measure absorbance immediately at 405 nm in a kinetic mode for 30-60 min at 37°C

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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the 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	Quantity	Storage temperature
Assay Buffer A1/Plasmin Assay Buffer	50 mL	-20°C or +4°C
Plasmin Substrate II/Plasmin Substrate	400 µL	-20°C
*pNA Standard I/p*NA Standard	20 µL	-20°C
Plasmin Positive Control	15 µL	-20°C
Plasmin Inhibitor Mix/Plasmin Inhibitor Mix (Lyophilized)	1 vial	-20°C

7. Materials Required, Not Supplied

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

- Multi-well spectrophotometer capable of measuring at OD 405 nm
- 96-well clear plate with flat bottom
- dH_2O

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.
- 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.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer A1/Plasmin Assay Buffer:**

Store at -20 °C or 4 °C. Bring to room temperature before use.

9.2 **Plasmin Substrate II/Plasmin Substrate:**

Store at -20 °C. Protect from light. Keep on ice while in use.

9.3 ***pNA Standard I/p*NA Standard:**

Store at -20 °C. Bring to room temperature before use.

9.4 **Plasmin Positive Control:**

Store at -20°C. Avoid repeated freeze-thaw cycles. Keep on ice while in use

9.5 **Plasmin Inhibitor Mix:**

9.5.1 Reconstitute using 30 µl dH_2O.

9.5.2 Pipette up and down to mix well.

9.5.3 Prepare a 40-fold dilution of the Plasmin Inhibitor Mix by adding 2 µl of the Stock Plasmin Inhibitor Mix into 78 µl Assay Buffer A1/Plasmin Assay Buffer.

Store at -20 °C. Keep on ice while in use.

ΔNote: Plasmin Inhibitor Mix must be prepared in dH_2O to preserve its activity.

Δ Note: Do not store the unused Diluted Inhibitor Mix. Prepare immediately before using.

10. Sample Preparation

10.1 Plasma

10.1.1 Collect whole blood into EDTA-treated tubes.
ΔNote: Plasma samples may need to be activated.

10.1.2 Remove cells from plasma by centrifugation for 10 min at 1,000-2,000 g using a refrigerated centrifuge.

10.1.3 Collect the supernatant.

10.1.4 Centrifuge for another 15 min at 2,000 g to deplete the platelets in the Plasma Sample.

10.1.5 Collect the supernatant and keep the Samples at 2-8 °C while handling.

10.1.6 Prepare a 20-fold dilution of the EDTA-treated Plasma in Assay Buffer A1/Plasmin Assay Buffer.

10.2 Plasmin Positive Control

10.2.1 Prepare 100-fold dilution of the Plasmin Positive Control by adding 2 µl of Plasmin Positive Control into 198 µl Assay Buffer A1/Plasmin Assay Buffer.

ΔNote: Do not store the diluted Plasmin Positive Control.

ΔNotes:

a. If the EDTA-treated plasma is not analyzed immediately, it should be stored at -80 °C or lower. It is important to avoid multiple freeze thaw cycles.

b. Heparin-treated plasma is not recommended for the assay. This is because Heparin acts as an uncompetitive inhibitor of the Plasmin Substrate II/plasmin substrate used in this kit.

c. Frozen/thawed Citrated-treated plasma generates Kallikrein activity which might affect the evaluation of plasmin activity.

11. Standard Curve

Prepare a 5 mM pNA Standard I/*p*NA Standard solution by adding 2 µl of 0.1 M pNA Standard I/*p*NA Standard into 38 µl Assay Buffer A1/Plasmin Assay Buffer.

11.1 Add 0, 2, 4, 6, 8, 10 µl of 5 mM (5 nmol/µl) pNA Standard I/*p*NA Standard into each well(s).

11.2 Adjust the volume to 100 µl/well with Assay Buffer A1/Plasmin Assay Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of pNA Standard I/*p*NA Standard.

Standard #	5 mM pNA Standard I/*p*NA Standard (µL)	Assay Buffer A1/Plasmin Assay Buffer (µL)	*p*NA (nmol/well)
1	0	100	0
2	2	98	10
3	4	96	20
4	6	94	30
5	8	92	40
6	10	90	50

11.3 Mix well and measure the absorbance at OD 405 nm.

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

12.1.1 Add 10-50 µl of diluted Plasma into duplicate wells of a 96-well clear plate labeled as Sample and Sample Background Control.

12.1.2 Add 4-10 µl of diluted Plasmin Positive Control into desired well(s).

12.1.3 Adjust the volume of Positive Control, Sample(s), and Sample Background Control to 60 µl/well with Assay Buffer A1/Plasmin Assay Buffer.

Inhibitor Mix

12.1.4 Add 20 µl of the Diluted Plasmin Inhibitor Mix (as prepared in step 9.5) to the Sample Background Control well(s).

12.1.5 Add 20 µl of Assay Buffer A1/Plasmin Assay Buffer to the Positive Control and Sample well(s).

12.1.6 Mix and incubate the plate at 37 °C for 30 min, avoid light.

Reaction Mix

12.1.7 Mix enough reagents for the number of assays to be performed. For each well, prepare 20 µl Reaction Mix containing:

Component	Volume to add per well
Assay Buffer A1/Plasmin Assay Buffer	16 µl
Plasmin Substrate II/Plasmin Substrate	4 µl

12.1.8 After 30 min incubation (see Step 12.1.4), add 20 µl Reaction Mix to Sample, Sample Background Control, and Positive Control wells.

12.1.9 Mix well.

ΔNote: The total volume of the Standard, Sample, Positive Control, Sample Background Control wells should be 100 µl/well.

12.1.10 Measure absorbance immediately at 405 nm in a kinetic mode for 30-60 min at 37°C.

– The pNA Standard I/pNA Standard can be read in End-point mode. For Samples, choose any two time points (t1 & t2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD1 and OD2).

13. Calculations

13.1 Subtract 0 Standard reading from all Standards readings.
13.2 Plot the pNA Standard Curve.
13.3 Calculate the signal from Plasmin in the Sample (ΔODS), (ΔODS = OD2 – OD1) and the Background signal from the Sample Background Control (ΔODBC):(ΔODBC = OD2 – OD1).
13.4 Subtract the Sample Background Control reading from its paired Sample reading (ΔODS-ΔODBC) and apply to the *p*NA Standard Curve to get B nmol of *p*NA generated during the reaction time (Δt = t2 - t1).

$$Sample\ Plasmin\ activity\ = \frac{B\ x\ B_{Sample\ Background\ Control}}{(\Delta t\ x\ V)}\ x\ D\ (nmol/min/mL)$$

B = pNA amount from Standard Curve (nmol)
V = Sample volume added into the reaction well (ml)
Δt = Reaction time (min)
D = Sample dilution factor (D = 1 for undiluted Samples)

Unit definition:

One unit of Plasmin activity is the amount of enzyme that releases 1.0 µmol of *p*NA per min at pH 8.4 at 37°C.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

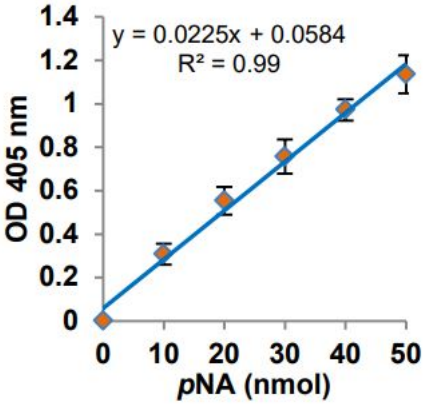

Figure 1. *p*NA Standard Curve.

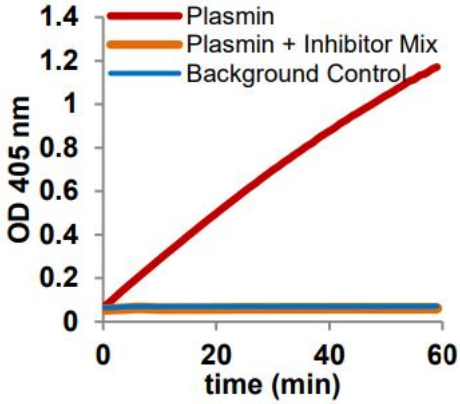

Figure 2. Measurement of purified Human Plasmin with or without the Plasmin Inhibitor Mix.

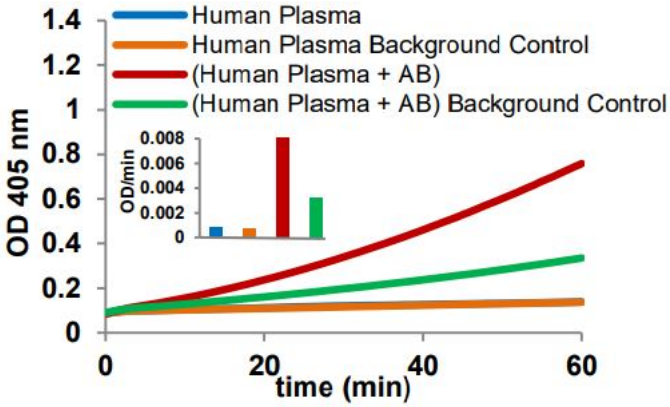

Figure 3. Measurement of Plasmin activity in Human Pooled EDTA-treated Plasma Sample (50 µl, 1:20 dilution) in the presence and absence of Activating Buffer containing Urokinase.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

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)