ab283368 – PAI1 Activity Assay Kit (Colorimetric)

For the screening of Plasminogen Activator Inhibitor-1.
For research use only - not intended for diagnostic use.

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 2 months.

Materials Supplied

Item	Quantity	Storage Condition
PAI-1 Assay Buffer	25 mL	-20°C
PAI-1 Acidification Buffer	5 mL	-20°C
PAI-1 Stop Buffer	5 mL	-20°C
Active Human tPA	1 vial	-20°C
Plasminogen	1 vial	-20°C
PAI-1 Substrate Mix	1 vial	-20°C
96-Well Half Area Plate	1 unit	-20°C

Materials Required, Not Supplied

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

- Multi-well spectrophotometer
- dH_2O
- Microplate shaker

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

PAI-1 Assay, Acidification and Stop Buffer: Warm to room temperature (RT) before use. Store at 4°C or -20°C.

Active Human tPA: Reconstitute with 100 µl PAI-1 Assay Buffer to prepare 5800 U/ml Active Human tPA stock solution. Pipette up and down to mix well. Divide into aliquots and store at -20°C. Keep on ice while in use.

Plasminogen: Reconstitute the vial with 44 µl PAI-1 Assay Buffer to prepare the Plasminogen stock solution. Store at -20°C. Keep on ice while in use.

PAI-1 Substrate Mix: Reconstitute the vial with 120 µl dH_2O. Divide into aliquots and store at -20°C. Keep on ice while in use.

96-Well Half Area Plate: Upon receiving, store the plate at RT.

Assay Protocol

Screening Compounds, Inhibitor Control & Background Control preparations:

40 U/ml tPA Solution Preparation: Prepare a 145-fold dilution of the Active Human tPA stock solution with PAI-1 Assay Buffer to make 40 U/ml tPA solution (i.e. add 2 µl of Active Human tPA stock solution into 288 µl PAI-1 Assay Buffer). Keep on ice while in use.

Sample, "0 U/ml PAI-1" & "40 U/ml PAI-1" Solution Preparation:
Plasma: Collect the citrate treated human plasma. Centrifuge the plasma at 5,000 x g for 10 min and 4°C to prepare the platelet poor plasma. Collect the supernatant and centrifuge again at 5,000 x g and 4°C for another 10 min. Collect the supernatant, which is the prepared plasma for the subsequent treatments. Store the prepared plasma at 2-8°C while in use. If

not used immediately, divide into aliquots and store the prepared plasma immediately at -70°C.

Cell Culture Supernatant: Grow the endothelial cells to confluency (usually 1-2 days). Collect the cell culture medium and centrifuge at 3,000 x g for 15 min and 4°C to remove any debris. Collect the cell supernatant. Mix 20 µl of prepared plasma or cell supernatant with 20 µl of 40 U/ml tPA in an Eppendorf tube labelled as "**Sample**". Mix 20 µl of 40 U/ml tPA with 20 µl of PAI-1 Assay Buffer in an Eppendorf tube labelled as "**0 U/ml PAI-1**". Add 40 µl of PAI-1 Assay Buffer in another Eppendorf tube labelled as "**40 U/ml PAI-1**". Incubate all the three Eppendorf tubes at RT for 30 min.

Δ Notes:
a. Citrate treated platelet poor plasma must be used for the assay. Platelet contamination may cause spurious results.
b. PAI-1 is unstable and must be processed and frozen within 3 hr of specimen collection.
c. There is large diurnal variation of PAI-1 activity in plasma, which should be taken into consideration when designing clinical studies
and routine applications. It is recommended that specimens should be the early morning fasting specimen.
d. If PAI-1 activity is above 40 U/ml, dilute the sample(s) with PAI-1 Assay Buffer and mark the dilution factor.

Acidification & Neutralization Step:

1. Add 40 µl of PAI-1 Acidification Buffer to all three Eppendorf tubes including **Sample**, **0 U/ml PAI-1** and **40 U/ml PAI-1**. Mix well and incubate at 37°C for 20 min, protected from light.
2. Add 80 µl of PAI-1 Assay Buffer to all the three tubes and mix well. The "**Sample**" tube is now ready for the assay.
3. Add 10 µl of the Sample into two parallel wells of a 96-Well Half Area Plate designed as "**PAI-1 Sample**" and "**PAI-1 Sample Background Control**".

Δ Note: Equilibrate the 96-well half area plate to 37°C before adding the Sample(s).

Standard Curve Preparation:

1. Intermediate "PAI-1" Standard Preparation: Prepare various intermediate PAI-1 Standards including 10 U/ml, 20 U/ml and 30 U/ml using PAI-1 Acidification Buffer treated 0 U/ml PAI-1 and 40 U/ml PAI-1 according to the table below. Mix well.

	0 U/ml PAI-1	40 U/ml PAI-1
10 U/ml PAI-1	45 µl	15 µl
20 U/ml PAI-1	30 µl	30 µl
30 U/ml PAI-1	15 µl	45 µl

2. Add 10 µl of 0 U/ml PAI-1, 10 U/ml PAI-1, 20 U/ml PAI-1, 30 U/ml PAI-1 and 40 U/ml PAI-1 Standards into wells of 96-Well Half Area Plate.

Reaction Mix Preparation:

1. Prepare a 25-fold dilution of the Plasminogen stock solution with PAI-1 Assay Buffer (i.e. add 4 µl of Plasminogen stock solution with 96 µl PAI-1 Assay Buffer).
2. Prepare 10-fold dilution of the reconstituted Substrate Mix with PAI-1 Assay Buffer (i.e. add 10 µl of reconstituted Substrate Mix with 90 µl PAI-1 Assay Buffer).
3. Prepare Reaction Mix (for both Standard(s) & PAI-1 Sample wells) and Background Mix (for PAI-1 Sample Background Control wells) according to the table below. Make sufficient amount of each type of mix to add 40 µl to all assay wells of that type.

Item	Reaction Mix	Background Mix
Diluted Plasminogen	10 µl	-- µl
Diluted Substrate Mix	10 µl	10 µl
PA-1 Assay Buffer	20 µl	30 µl

4. Mix well. Add 40 µl of Reaction Mix to PAI-1 Standard(s) & PAI-1 Sample wells and 40 µl of Background Mix to PAI-1 Sample Background Control well(s). Mix well and incubate at 37°C for 90 min, protected from light. The total volume of each well is 50 µl.
5. After 90-min incubation, add 50 µl of PAI-1 Stop Buffer to all wells containing PAI-1 Sample(s), PAI-1 Sample Background Control and PAI-1 Standards. Shake plate for 1 minute on a microplate shaker at 250 RPM to mix.

Δ Note: Equilibrate the PAI-1 Stop Buffer to 37°C before adding to the wells.

Measurement

Measure the colorimetric signal (OD 405 nm) at 37°C in end-point mode.

Calculation:

1. Subtract the "40 U/ml PAI-1" Standard reading from all Standards readings.
2. Plot the PAI-1 Standard Curve.
3. Subtract the PAI-1 Sample Background Control reading from all PAI-1 Sample readings to get the corrected PAI-1 Sample readings.
4. Apply the corrected PAI-1 Sample readings to the PAI-1 Standard Curve to obtain the corresponding PAI-1 activity (U/ml) as:

Sample PAI-1 Activity = B * D = U/ml

Where:

B = PAI-1 activity from the Standard Curve (U/ml)

D = Sample dilution factor (D=1 for undiluted Sample(s))

Unit Definition: One unit of PAI-1 activity was defined as the amount of PAI-1 that inhibits one unit of tPA activity under Assay conditions.

Technical Support

Copyright © 2026 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

For all 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)

Version 5a, Last updated February 4, 2026