

**ab108894**

**Plasminogen activator  
Inhibitor Type 1 Human  
Chromogenic Activity Kit**

**Instructions for Use**

For the quantitative measurement of Human Type 1 plasminogen activator inhibitor (PAI-1) activity in cell culture supernatants, serum and plasma samples.

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

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# 1. Introduction

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Type I plasminogen activator inhibitor (PAI-1) is a 50 kDa serpin family member that inhibits tissue- and urokinase-type plasminogen activators (t-PA, u-PA). This protein appears to be an important regulator of plasminogen activation by t-PA and extracellular proteolysis by u-PA. The plasminogen activator proteolytic enzyme systems are important not only for fibrinolysis but also for extracellular matrix remodeling, and have been implicated in a number of normal and pathological processes including angiogenesis, ovulation and embryogenesis, thrombotic and hemorrhagic disorders, connective tissue diseases, neoplasm and sepsis. PAI-1 is a prognosticator in breast cancer, gastric cancer, various forms of lung cancer and cervical cancer.

ab108894 Human PAI-1 Chromogenic Activity Assay Kit is developed to determine human PAI-1 activity in plasma, serum and cell culture supernatants. A fixed amount of tPA is added in excess to undiluted sample, which allows PAI-1 and tPA to form an inactive complex. The assay measures plasminogen activation by residual tPA in coupled assays that contain tPA, plasminogen, and a plasmin-specific synthetic substrate. The amount of plasmin produced is quantitated using a highly specific plasmin substrate releasing a yellow paranitroaniline (pNA) chromophore. The absorbance of the pNA at 405 nm is inversely proportional to the PAI-1 enzymatic activity.

## 2. Assay Summary

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Prepare all reagents, samples and standards as instructed.



Add 80  $\mu$ l Assay mix to each well.



Add 20  $\mu$ l Standards or samples to each well.



Incubate at 37°C.



Reading at 405nm every hour for 8 – 10 hours.

Cover and incubate at 37°C after each reading.

## 3. Kit Contents

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Item	Amount	Storage Condition (Before Preparation)
Microplate (12 x 8 well strips)	96 wells	4°C
tPA Standard (Lyophilized)	1 vial	-20°C
Assay Diluent	30 mL	4°C
Human Plasminogen (Lyophilized)	3 vials	-20°C
Plasmin Substrate (Lyophilized)	2 vials	-20°C
Sealing Tapes	3	N/A

## **4. Storage and Handling**

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Upon arrival, immediately store components of the kit at recommended temperatures.

Unused microplate wells may be returned and resealed.

Opened Assay Diluent may be stored for up to 30 days at 2-8°C.

## **5. Additional Materials Required**

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- Microplate reader capable of measuring absorbance at 405nm.
- Precision pipettes to deliver 1 µl to 1 ml volumes.
- Distilled or deionized reagent grade water.
- Incubator (37°C)

## 6. Reagent Preparation

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**Plasminogen (1X):** Add 1.2 mL reagent grade water to generate a 1X stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and used within 48 hours.

**Plasmin Substrate (1X):** Add 0.55 ml of reagent grade water to generate a 1x stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

## 7. Standard Preparation

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Reconstitute the Human tPA Standard (100 IU) with 5 mL of Assay Diluent to generate a 20 IU/mL standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (20 IU/mL) with Assay Diluent to produce 10, 5, 2.5, 1.25, and 0.625 IU/mL solutions. Assay Diluent serves as the zero standard (0 IU/mL). Any remaining solution should be frozen at -20°C and used within 30 days.

<b>Standard Point</b>	<b>Dilution</b>	<b>[tPA] (IU/mL)</b>	<b>*[PAI-1] (AU/mL)</b>
P1	Standard Solution (20 IU/mL)	20	0
P2	1 part P1 + 1 part Assay Diluent	10	0.625
P3	1 part P2 + 1 part Assay Diluent	5	1.250
P4	1 part P3 + 1 part Assay Diluent	2.5	2.50
P5	1 part P4 + 1 part Assay Diluent	1.25	5.000
P6	1 part P5 + 1 part Assay Diluent	0.625	10.00
P7	Assay Diluent	0	20.00

\*Note: One arbitrary unit (AU) of inhibitor is defined as the amount that inhibits one IU of tPA/mL under the testing conditions.

## 8. Sample Preparation

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**Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes to obtain platelet-poor plasma. A 2-fold sample dilution is suggested into Assay Diluent; however, user should determine optimal dilution factor depending on application needs. Mix equal volumes of the diluted plasma sample with the diluted tPA Standard solution (20 IU/mL) resulting in a 4-fold dilution. The time of plasma collection should be standardized as PAI-1 levels show the marked diurnal variation (EDTA or Heparin can also be used as an anticoagulant). The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

**Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes, and remove serum. A 2-fold sample dilution is suggested into Assay Diluent; however, user should determine optimal dilution factor depending on application needs. Mix equal volumes of the diluted serum sample with the diluted tPA Standard solution (20 IU/mL) resulting in a 4-fold dilution. The time of serum collection should be standardized as PAI-1 levels show the marked diurnal variation. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

**Cell culture supernatants:** Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris. Collect supernatants and dilute samples in Assay Diluent if necessary. Next, dilute supernatants with an equal volume of the tPA Standard solution (20 IU/mL). We advise to determine the best dilution for every supernatant individually. Avoid repeated freeze-thaw cycles.

## 9. Assay Method

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1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at 37°C in a humid incubator. Remove excess microplate strips from the plate frame.
2. Assay Mix: Freshly prepare the desired volume of the Assay Mix by combining the following reagents according to the assay numbers (n). It is recommended that the Assay mixture is made in 10% excess.

<u>Reagents</u>	<u>n=1</u>
Assay Diluent	65 $\mu$ L
Plasminogen	5 $\mu$ L
Plasmin Substrate	10 $\mu$ L

3. Add 80  $\mu$ L of the Assay Mix to each well. Gently tap plate to thoroughly coat the wells. Add 20  $\mu$ L of tPA Standard or sample to each well. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Read the absorbance at 405nm for a zero-minute background reading. Cover the wells with a sealing tape and incubate at 37° C in a humid incubator to avoid evaporation. Incubate microplate at 37° C after each reading.
4. Read the absorbance at 405nm at 0 minutes then every 1 hour for 8 – 10 hours after. Cover wells with a sealing tape and incubate at 37°C after each reading.

## 10. Data Analysis

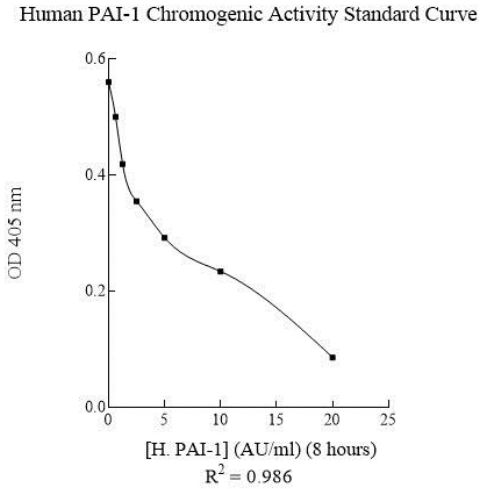
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Calculate the mean value of the triplicate for each standard and sample. Plot absorbance vs time for each standard and sample. The linear part of the plot will correspond to the initial reaction rate, when substrate is still highly abundant. Pick a time point where standards and samples are in their linear range. Use the data from that time point or range of time points to create the standard curve.

To generate a Standard Curve from the optimal reaction time frame, plot the graph using the standard concentrations on the x-axis and the corresponding mean 405 nm absorbance (OD) or change in absorbance per minute ( $\Delta A/\text{min}$ ) on the y-axis. The best-fit line can be determined by regression analysis of the 4-parameter curve. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor

## Typical Data

The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



## Sensitivity

The minimum detectable dose of PAI-1 is typically 0.56 AU/ml.

## **11. Specificity**

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No significant cross-reactivity or interference was observed.

## 12. Notes

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