AssaySense

Human PAI-1 Chromogenic Activity Kit

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For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Step 1. Add 80 µl of Assay Mix per well.
Add 20 µl of Standard or Sample per well.

Step 2. Read the absorbance at 405 nm for a zero minute background reading.
Cover and incubate at 37°C.

Step 3. Read every hour for 8 – 10 hours at 405 nm.
Cover and incubate at 37°C after each reading.

Symbol Key

Consult instructions for use.

Assay Template

	A	B	C	D	E	F	G	H
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AssaySense Human Plasminogen Activator Inhibitor-1 (PAI-1) Chromogenic Activity Kit

Catalog No. CP1100

Sample insert for reference use only

Introduction

Type I plasminogen activator inhibitor (PAI-1) is a 50 kDa serpin family member that inhibits tissue- and urokinase-type plaminogen activators (tPA, uPA). This protein appears to be an important regulator of plasminogen activation by tPA and extracellular proteolysis by uPA (1-3). 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 angiogensis, ovulation and embryogenesis, thrombotic and hemorrhagic disorders, connective tissue diseases, neoplasm, and sepsis (4-6). PAI-1 is a prognosticator in breast cancer, gastric cancer, various forms of lung cancer and cervical cancer (7-9).

Principle of the Assay

The AssaySense Human PAI-1 Chromogenic Activity Kit is developed to determine PAI-1 activity in human **plasma, serum, and cell culture samples**. This kit is also validated for use with **canine, bovine, equine, swine, and rabbit samples.** A fixed amount of tPA is added in excess to the diluted 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 para-nitroaniline (pNA) chromophore. The absorbance of the pNA at 405 nm is inversely proportional to the PAI-1 enzymatic activity.

Caution and Warning

- This product is for **Research Use Only** and is not intended for use in diagnostic procedures.
- Prepare all reagents, as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- The kit should not be used beyond the expiration date.

Reagents

The activity assay kit contains sufficient reagents to perform 96 tests using the microplate method.

- **Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells).
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human tPA Standard:** Lyophilized (52 IU).
- **Assay Diluent (1x):** Buffered protein base (30 ml).
- **Human Plasminogen:** Lyophilized, 3 vials.
- **Plasmin Substrate:** Lyophilized, 2 vials.

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store Standard, Plasminogen, and Plasmin Substrate at -20°C.
- Store Microplate and Assay Diluent at 2-8°C.
- Unused microplate wells may be returned to the pouch and resealed.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel)
- Deionized or distilled reagent grade water
- Incubator (37°C)

Sample Collection, Preparation, and Storage

- **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. Next, mix equal volume of the 2-fold diluted sample with the 2-fold diluted **Human tPA Standard (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. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **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. Next, mix equal volume of the 2-fold diluted sample with the 2-fold diluted **Human tPA Standard (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. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- **Cell Culture Supernatant:** Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. If necessary, dilute samples into Assay Diluent; user should determine optimal sample dilution depending on application needs. Next, mix equal volume of the (un)diluted sample with the 2-fold diluted **Human tPA Standard (20 IU/ml)**, resulting in a dilution factor that is 2-fold of your sample dilution. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater (*for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
A) 4 µl sample : 396 µl buffer (100x) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*
1000x	**100000x**
A) 4 µl sample : 396 µl buffer (100x) B) 24 µl of A : 216 µl buffer (10x) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) C) 24 µl of B : 216 µl buffer (10x) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **Human tPA Standard:** Reconstitute the Human tPA Standard (52 IU) with 1.3 ml of Assay Diluent to generate a 40 IU/ml standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making

dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (40 IU/ml) 2-fold with equal volume of Assay Diluent to produce 20, 10, 5, 2.5, 1.25, and 0.625 IU/ml solutions. Assay Diluent serves as the zero standard (0 IU/ml). Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

Standard Point	Dilution	[tPA] (IU/ml)	*[PAI-1] (AU/ml)
P1	1 part Standard (40 IU/ml) + 1 part Assay Diluent	20	0.0
P2	1 part P1 + 1 part Assay Diluent	10	0.625
P3	1 part P2 + 1 part Assay Diluent	5.0	1.25
P4	1 part P3 + 1 part Assay Diluent	2.5	2.5
P5	1 part P4 + 1 part Assay Diluent	1.25	5.0
P6	1 part P5 + 1 part Assay Diluent	0.625	10
P7	Assay Diluent	0.0	20

- **Plasminogen:** Add 1.2 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. 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:** 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.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at 37°C in a humid incubator to avoid evaporation.
- Remove excess microplate strips from the plate frame.
- Freshly prepare the desired volume of the Assay Mix by combining the following reagents according to the number of wells in the assay (n) plus one well.

Assay Mix Reagent	n = 1 well
Assay Diluent	65 µl
Plasminogen	5 µl
Plasmin Substrate	10 µl

- Add 80 µl of Assay Mix to each well. Gently tap plate to thoroughly coat the wells. Add 20 µl of Human 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 405 nm for a zero minute background reading. Cover wells with a sealing tape and incubate at 37°C in a humid incubator.

- Read the absorbance at 405 nm every hour for 8 – 10 hours. Cover wells with a sealing tape and incubate at 37°C after each reading.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve from the optimal reaction time, 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 (ΔA/min) on the y-axis after subtracting the background. 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.

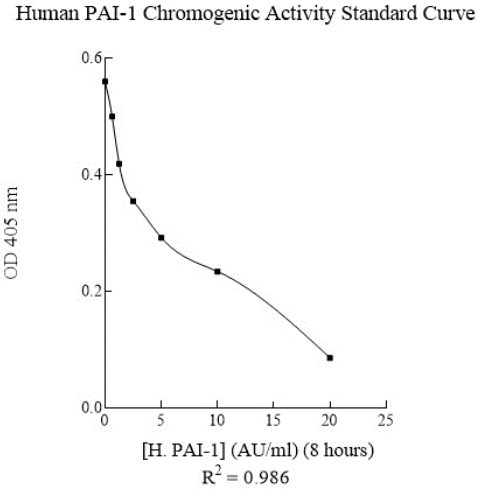
Typical Data

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	AU/ml	Average OD
P1	0.0	0.560
P2	0.625	0.500
P3	1.25	0.419
P4	2.5	0.355
P5	5.0	0.292
P6	10	0.234
P7	20	0.086

Standard Curve

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



Performance Characteristics

- The minimum detectable dose of human PAI-1 at 8 hours as calculated by 2SD from the mean of a zero standard was established to be 0.56 AU/ml.

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of improper components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	• Each step of the procedure should be performed uninterrupted.
	Omission of step	• Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	• Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	• Check pipette calibration. • Check pipette for proper performance.
	Improper reagent preparation	• Consult reagent preparation section for the correct dilutions of all reagents.

	Insufficient or prolonged incubation periods	• Consult the provided procedure for correct incubation time.
Deficient Standard Curve Fit	Non-optimal sample dilution	• User should determine the optimal dilution factor for samples.
	Contamination of reagents	• A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	• Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.

References

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