Human PAI-1
Activity Kit

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Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

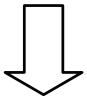
The datasheet is only a first information.
The relevant datasheet is included with the product.

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

Add 80 µl of Assay Mix
and 20 µl of standard/sample per well.
Incubate at 37°C.



Read every 1 hour for 8 hours at 405 nm.

Assay Template

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								

AssaySense Human PAI-1 Chromogenic Activity Kit

Catalog No. CP1100
Sample Insert/Reference Only

Introduction

Type I plasminogen activator inhibitor (PAI-1) is a 50 kDa serpin family member that inhibits tissue- and urokinase-type plasminogen 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 angiogenesis, ovulation and embryogenesis, thrombotic and hemorrhagic disorders, connective tissue diseases, neoplasm, and sepsis (4, 5). PAI-1 is a prognosticator in breast cancer (6), gastric cancer (7), various forms of lung cancer (8), and cervical cancer (9).

Principle of Assay

The AssaySense Human PAI-1 Chromogenic Activity Kit is developed to determine human PAI-1 activity in plasma and cell culture supernatant 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

- **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 protocol. However, the user should determine the optimal dilution factor.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- All human source materials have been tested and found to be negative to HbsAg, HIV-1 and HCV by FDA approved methods.

Reagents

The activity assay kit contains sufficient reagents to perform 100 tests using microplate method.

- **Microplate:** One 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.
- **Assay Diluent:** 30 ml
- **Human tPA Standard:** 2 vials, lyophilized (48 IU)
- **Human Plasminogen:** 2 vials, lyophilized
- **Plasmin Substrate:** 2 vials, lyophilized

Storage Condition

- Store components of the kit 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 and resealed.
- Opened Assay Diluent may be stored for up to 30 days at 2-8°C.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes (1-20 μl, 20-200 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. Dilute plasma 1:4 with Assay Diluent. Next, mix equal volumes of diluted samples (ex. 15 μl) with 40 IU/ml tPA Standard (ex. 15 μl) for 10 minutes at room temperature prior to the assay. 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).
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and dilute samples if necessary. Next, mix equal volumes of sample (ex. 15 μl) with 40 IU/ml tPA Standard (ex. 15 μl) for 10 minutes at room temperature prior to the assay.

Reagent Preparation

- **Standard Curve:** Reconstitute the Human tPA Standard (48 IU) with 1.2 ml of Assay Diluent to generate a standard solution of 40 IU/ml. 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 (40 IU/ml) with 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 solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[tPA] (IU/ml)	*[PAI-1] (AU/ml)
P1	1 part Standard (40 IU/ml) + 1 part Assay Diluent	20.00	0.00
P2	1 part P1 + 1 part Assay Diluent	10.00	0.625
P3	1 part P2 + 1 part Assay Diluent	5.000	1.250
P4	1 part P3 + 1 part Assay Diluent	2.500	2.500
P5	1 part P4 + 1 part Assay Diluent	1.250	5.000
P6	1 part P5 + 1 part Assay Diluent	0.625	10.00
P7	Assay Diluent	0.000	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.

- **Plasminogen:** Add 1.2 ml reagent grade water. Any remaining solution should be frozen at -20°C and used within 30 days.
- **Plasmin Substrate**: Add 0.55ml reagent grade water. Any remaining solution should be frozen at -20°C and used within 30 days.

Assay Procedure

- Assay Mix: Freshly prepare the desired volume of the Assay Mix by combining the following reagents according to the assay numbers (n).

Reagents	n=1
Assay Diluent	60 µl
Plasminogen	10 µl
Plasmin Substrate	10 µl

- Add 80 µl of the above Assay Mix to each well.
- Add 20 µl of tPA Standard or sample per well and mix gently.
- Incubate at 37°C, and read the absorbance at 405 nm every hour for eight hours.

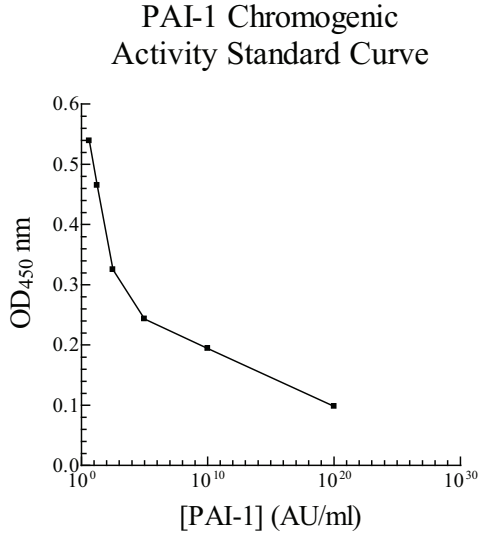
Assay Mix	80 µl
tPA Standard or Sample	20 µl
Cover wells with sealing tape. Incubate 37°C. Read the absorbance at 405 nm every hour for eight hours.	

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve from the initial reaction time, plot the graph using the standard concentrations on the x-axis and the corresponding mean 405 nm absorbance or change in absorbance per minute (ΔA/min) on the y-axis. The best-fit line can be determined by regression analysis of the linear portion of the curve.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

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 PAI-1 is typically ~ 0.6 IU/ml.
- Intra-assay and inter-assay coefficients of variation were 4.7 % and 7.0% respectively.
- No significant cross-reactivity or interference was observed.

References

(1) Sprengers, E.D (1987) *Blood* 190:381
(2) Loskutoff, D.J (1989) *Progress in Haemost. Thromb.* 91989:87.
(3) Schneideman, J (1991) *Trends Cardiovascular Med.* 1:99
(4) Dano, K *et. al.* (1985) *Adv. Cancer Res.* 44:139
(5) Vassalli, J.D. (1991) *J. Clin. Inves*. 88:1067
(6) Dano, K. *et. al.* (1985) *Cancer Res.* 44:139
(7) He, C *et al* (1989) *Proc. Natl. Acad. Sci.* 86, 2632
(8) Pedersen, H *et al.* (1994) *Cancer Res.* 54:120
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