AssaySense Human PAI-1 Chromogenic Activity Assay Kit

Catalog Number CP1100

Introduction

Type I plasminogen activator inhibitor (PAI-1) is a 50 kDa serpin family member that inhibits tissue- and urokinase-type plaminogen 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 (1, 2, 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, 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 AngioSense Human PAI-1 Chromogenic Activity Assay Kit is developed to determine human PAI-1 activity in plasma, cell culture supernatants and tissue. A fixed amount of tPA is added in excess to undiluted plasma, 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, firbinogen fragments, plasminogen, and a plsmin-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 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 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Assay Diluent:** 30 ml
- **tPA Standard:** 2 vials human tPA (88 IU)
- **Human Fibriongen Fragments:** 1 vial
- **Human Plasminogen:** 1 vial
- **Plasmin Substrate:** 2 tubes
- **Substrate Diluent:** 5 ml

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8^{0}C. Store reconstituted standard and reagents at -20^{0}C or below.

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^{0}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 4,000 x g for 10 minutes to obtain platelet-poor plasma. Dilute plasma 1:4 with Assay Diluent. Mix equal volumes of diluted samples (20µl) with 40 IU/ml tPA (20µl) 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.

- **Cell Culture Supernatants:** Centrifuge cell culture media at 3,000 x g for 10 minutes to remove debris. Collect supernatants and mix samples 1:1 with 40 IU/ml tPA for 10 minutes at room temperature prior to the assay.

- **Tissue:** Extract tissue samples with 0.1 M Tris-buffered saline (pH7.4) containing 0.5% Triton X-100 and centrifuge at 14,000 x g for 30 min. Collect the supernatant and measure the protein concentration. Mix samples 1:1 with 40 IU/ml tPA for 10 minutes at room temperature prior to the assay.

Reagent Preparation

- **Standard Curve:** Reconstitute the tPA Standard (88 IU) with 2.2 ml of Assay Diluent to generate a solution of 40 IU/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (40 IU/ml) with desired volume of Assay Diluent to produce 30, 20, and 10 IU/ml. Assay Diluent serves as the zero standard (0 IU/ml).

Standard Point	Dilution	[tPA] (IU/ml)	*[PAI-1] (AU/ml)
P1	Standard (40 IU/ml)	40.00	0.00
P2	75 µl P1 + 25 µl Assay Diluent	30.00	10.00
P3	50 µl P1 + 50 µl Assay Diluent	20.00	20.00
P4	25 µl P1 + 75 µl Assay Diluent	10.00	30.00
P5	Assay Diluent	0.00	40.00

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

- **Fibrinogen Fragments:** Add 1.2 ml Assay Diluent.
- **Plasminogen:** Add 1.1 ml Assay Diluent.
- **Plasmin Substrate**: Add 1.1 ml reagent grade water.

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	10 µl
Fibrinogen Fragments	10 µl
Plasminogen	10 µl

- Add 30 µl of the above Assay Mix to each well of the 96-well plate.
- Add 10 µl of tPA Standards or testing samples per well and mix gently. Incubate at 37^0C for 30 min.
- Add 20 µl of Plasmin Substrate to each well and mix gently. Incubate at 37^0C for three hours depending on the PAI-1 level measured, and read the absorbances at 405 nm periodically every 60 minutes.

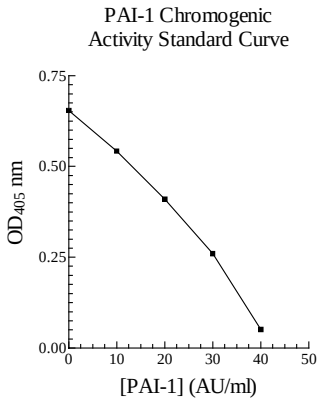
Assay Mix	30 µl
tPA or Samples	10 µl
37^0C, 30 minutes	
Plasmin Substrate	20 µl
37^0C, read the absorbances at 405 nm every one hour for three hours	

Data Analysis

- Calculate the mean value of the triplicate 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 < 5 AU/ml.
- Intra-assay and inter-assay coefficients of variation were 5.1 % and 8.8% respectively.
- No significant cross-reactivity or interference was observed.

References

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