AssayMax Human Plasminogen Activator Inhibitor-1 (PAI-1) ELISA Kit

Catalog Number EP1100-1
Lot #

Introduction

Type I plasminogen activator inhibitor (PAI-1) is a 43 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 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).

Principal of the Assay

The AssayMax Human PAI-1 ELISA kit is designed for detection of PAI-1 in human plasma, tissue extracts or cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures PAI-1 in 3.5 hours. A murine antibody specific for PAI-1 has been pre-coated onto a microplate. PAI-1 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for PAI-1, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **PAI-1 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a murine antibody against PAI-1.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **PAI-1 Standard:** Recombinant human PAI-1 in a buffered protein base (15 ng, lyophilized).

- **Biotinylated PAI-1 Antibody (100x):** A 100-fold biotinylated polyclonal antibody against PAI-1 (80 µl).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (120 µl).
- **Sample Diluent Concentrate (8x)**: A 8-fold buffered protein base (15 ml).
- **Assay Diluent:** A buffered protein base (20 ml).
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

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 at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, and multiple channel pipettes)
- Deionized or distilled reagent grade water

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 4,000x g for 10 minutes to obtain platelet-poor plasma. Dilute sample supernatants 1:20 with Sample Diluent and assay. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles. 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 2,000x g for 10 minutes to remove debris. Collect supernatants and assay. The samples can be stored at -20^{0}C or below. Avoid repeated freeze-thaw cycles.
- **Tissue:** Extract tissue samples with 50 mM Tris-buffered saline (pH7.4) containing 0.5% Triton X-100 and centrifuge at 14,000x g for 30 min. Collect the supernatant and measure the protein concentration. Dilute the tissue extract 1:20 into Sample Diluent and assay. The undiluted samples can be stored at -20^{0}C or below.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **Standard Curve:** Reconstitute the 15 ng of human PAI-1 Standard with 3 ml of Sample Diluent to generate a stock solution of 5 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the PAI-1 standard solution (5 ng/ml) twofold with equal volume of Sample Diluent to produce 5, 2.5, 1.25, 0.625, 0.313, 0.156 and 0.078 ng/ml. Sample Diluent serves as the zero standard (0 ng/ml).

Standard Point	Dilution	[PAI-1] (ng/ml)
P1	Standard (5 ng/ml)	5.000
P2	1 part P1 + 1 part Sample Diluent	2.500
P3	1 part P2 + 1 part Sample Diluent	1.250
P4	1 part P3 + 1 part Sample Diluent	0.625
P5	1 part P4 + 1 part Sample Diluent	0.313
P6	1 part P5 + 1 part Sample Diluent	0.156
P7	1 part P6 + 1 part Sample Diluent	0.078
P8	Sample Diluent	0.000

- **Sample Diluent Concentrate (8x):** Dilute Sample Diluent 1:8 with reagent grade water.
- **Biotinylated PAI-1 Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with Assay Diluent.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer 1:10 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with Assay Diluent.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Biotinylated PAI-1 Antibody to each well and incubate for 30 minutes.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Chromogen Substrate per well and incubate for approximately 10 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

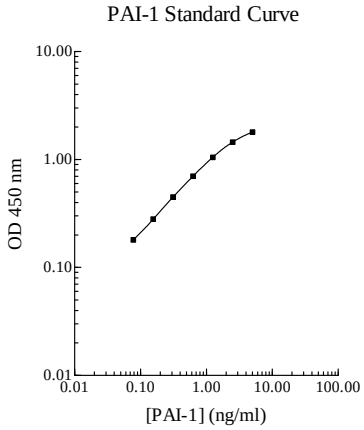
Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.
- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using 4-parameter or log-log curve fit.

- Determine the unknown sample concentration from the Standard Curve and multiply the plasma or tissue value by the dilution factor of 20.

Standard Curve

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



Sensitivity and Specificity

- The minimum detectable dose of PAI-1 is typically < 50 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.7% and 8.3% respectively.
- This assay recognizes both natural and recombinant human PAI-1.

Reference Values

Normal human platelet-poor plasma concentration of PAI-1 has been reported to range from 5 to 40 ng/ml (10). The variability was due in part to the marked diurnal variation on PAI-1, with lower values in the afternoon than in the morning, and also to age-related changes.

References

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