



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

Catalog Number EP1100-1

Introduction

Type I plasminogen activator inhibitor (PAI-1) is a 43 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 (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 4 hours. A polyclonal 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 polyclonal 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 (60 ng, lyophilized).
- **Biotinylated PAI-1 Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against PAI-1 (80 μ l).

- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 μ l).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store unopened kit at 2-8⁰C up to expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8⁰C. Store reconstituted reagents at -20⁰C 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 μ l, 20-200 μ l, 200-1000 μ l 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 4000 x g for 10 minutes to obtain platelet-poor plasma. Dilute sample supernatants 1:4 with MIX Diluent and assay. The undiluted samples can be stored at -20⁰C 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 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. The samples can be stored at -20⁰C 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 14000 x g for 30 min. Collect the supernatant and measure the protein concentration. Dilute the tissue extract 1:4 into MIX Diluent and assay. The undiluted samples can be stored at -20⁰C or below.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **MIX Diluent Concentrate (10x):** Dilute MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8⁰C.
- **Standard Curve:** Reconstitute the 60 ng of human PAI-1 Standard with 3 ml of MIX Diluent to generate a 20 ng/ml of standard solution. 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 twofold with equal volume of MIX Diluent to produce 20, 10, 5, 2.5, 1.25, 0.625 and 0.313 ng/ml. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20⁰C.

Standard Point	Dilution	[PAI-1] (ng/ml)
P1	Standard (20 ng/ml)	20.000
P2	1 part P1 + 1 part MIX Diluent	10.000
P3	1 part P2 + 1 part MIX Diluent	5.000
P4	1 part P3 + 1 part MIX Diluent	2.500
P5	1 part P4 + 1 part MIX Diluent	1.250
P6	1 part P5 + 1 part MIX Diluent	0.625
P7	1 part P6 + 1 part MIX Diluent	0.313
P8	MIX Diluent	0.000

- **Biotinylated PAI-1 Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

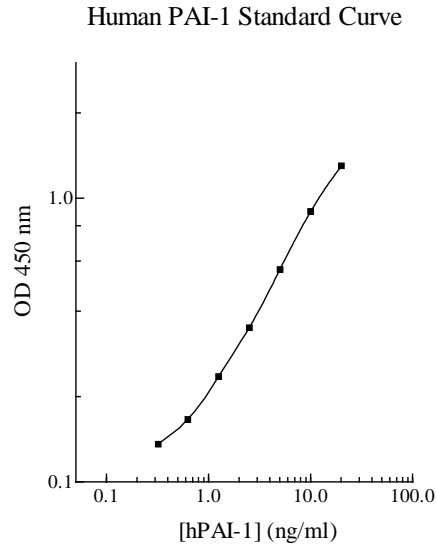
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal 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 with a sealing tape 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 completely remove liquid at each step.
- Add 50 µl of Biotinylated PAI-1 Antibody to each well and incubate for one hour.
- 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 12 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 optical densities to reduce the readings after stopping the reaction for about 10 minutes.

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 log-log or four-parameter logistic curve-fit.
- 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 < 200 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.0% and 7.0% respectively.
- This assay recognizes both natural and recombinant human PAI-1.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Tissue Extract
1:2	95%	97%
1:4	105%	103%
1:8	105%	105%

Sample Dilution	Average Percentage of Expected Value
	Cell Culture Media
1:2	100%
1:4	98%
1:8	101%

Recovery

Standard Added Value	1 - 10 ng
Recovery %	80-120 %
Average Recovery %	100 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	<20 (suggest 1:2 dilution for plasma)
Monkey	> 60 (suggest 1:5 dilution for plasma)
Mouse	<1
Rat	<10 (suggest no dilution for plasma)
Swine	<10 (suggest no dilution for plasma)

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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