



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

Catalog No. 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, serum, tissue extracts, saliva, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures PAI-1 in less than 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

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-antibody, and SP conjugate) 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.**
- **Spin down the SP conjugate vial and the biotinylated-antibody vial before opening and using contents.**
- 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:** Human PAI-1 in a buffered protein base (10 ng, lyophilized).
- **Biotinylated PAI-1 Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against PAI-1 (140 µl).
- **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, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **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 components of the kit at 2-8⁰C or -20⁰C upon arrival up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20⁰C
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8⁰C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8⁰C.
- Store Standard at 2-8⁰C before reconstituting with Diluent and at -20⁰C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, 200-1000 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 4000 x g for 10 minutes to obtain platelet-poor plasma. Dilute sample supernatants 1:10 with MIX Diluent and assay. The undiluted samples can be stored for up to 3 months at -20⁰C or below -20⁰C. Avoid repeated freeze-thaw cycles. The time of plasma collection should be standardized as PAI-1 levels show the marked diurnal variation.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum, dilute samples 1:10 into MIX Diluent and assay. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **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, measure the protein concentration, and assay. The undiluted samples can be stored at -20⁰C or below.
- **Saliva:** Collect saliva using sample tube. Centrifuge samples at 600 x g for 10 minutes and assay. Store samples at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C.
- **Standard Curve:** Reconstitute the 10 ng of human PAI-1 Standard with 2 ml of MIX Diluent to generate a stock standard solution of 5 ng/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 PAI-1 standard solution 1:2 with equal volume of MIX Diluent to produce 2.5, 1.25, 0.625, 0.313, 0.156, and 0.078 ng/ml. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C.

Standard Point	Dilution	[PAI-1] (ng/ml)
P1	Standard (10 ng/ml) + 1 part MIX Diluent	5.000
P2	1 part P1 + 1 part MIX Diluent	2.500
P3	1 part P2 + 1 part MIX Diluent	1.250
P4	1 part P3 + 1 part MIX Diluent	0.625
P5	1 part P4 + 1 part MIX Diluent	0.313
P6	1 part P5 + 1 part MIX Diluent	0.156
P7	1 part P6 + 1 part MIX Diluent	0.078
P8	MIX Diluent	0.000

- **Biotinylated PAI-1 Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. 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 manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 µl of Biotinylated PAI-1 Antibody to each well and incubate for one hour.
- Wash the microplate as described 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 the microplate as described above.

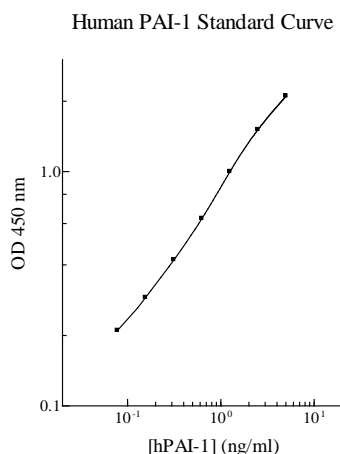
- Add 50 µl of Chromogen Substrate per well and incubate for approximately 8 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. 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 duplicate or 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 ~ 0.07 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.7% and 7.2% respectively.
- This assay recognizes both natural and recombinant human PAI-1.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:5	97%	95%
1:10	101%	99%
1:20	105%	106%

	Average Percentage of Expected Value
Sample Dilution	Saliva
No dilution	97%
1:2	100%
1:4	103%

Recovery

Standard Added Value	0.3-3 ng/ml
Recovery %	88-109 %
Average Recovery %	98 %

Cross-Reactivity

Species	% Cross Reactivity
Canine	1%
Monkey	20%
Mouse	2%
Rat	20%
Swine	None
Rabbit	20%
Bovine	5%

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

- (1) Sprengers, E.D. (1987) *Blood* 190:381
- (2) Loskutoff, D.J. (1989) *Progress in Haemost. Thromb.* 9:1989:87.
- (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
- (9) Kobayashi, H. *et al.* (1994) *Cancer Res.* 54:6539
- (10) Booth, NA *et al.* (1988) *Br. J. Haematology* 70:327

Version 9.3

Related products

- EP1105-1 AssayMax Human PAI-1/tPA ELISA Kit (Plasma, Cell Culture Supernatants, and Tissue samples)
- ET1001-1 AssayMax Human tPA ELISA Kit (Plasma, Urine, Saliva, Cell Culture Supernatants, and Tissue samples)