AssayMax Human PAI-1/tPA ELISA Kit

Catalog Number EP1105-1

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). tPA is a 68 kDa serine protease that converts the plasminogen into plasmin and facilitates the digestion of fibrin clots (1, 2). In plasma, half or more of PAI-1 and most tPA present in the circulation is in an inhibited complex (3). In the resting state in healthy individuals, typically less than 20% of tPA is present in its free form in the plasma. In normal individuals as well as in patients with recurrent venous thrombosis, high PAI-1 plasma concentration is usually associated with high tPA antigen (but not with free tPA) levels (4). PAI-1/tPA complex, a novel fibrinolytic marker, increases during the pregnancy-associated hypercoagulable state, atherosclerosis and vascular spasm (5). Determination of PAI-1/tPA complex may provide valuable prognostic information with respect to breast cancer patients (6) and myocardial infarction in patients with manifest coronary heart disease (7).

Principal of the Assay

The AssayMaxHuman PAI-1/tPA ELISA kit is designed for detection of PAI-1/tPA in human plasma, tissue extracts or cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures PAI-1/tPA in 3.5 hours. A murine antibody specific for PAI-1 has been pre-coated onto a microplate. PAI-1/tPA complex in standards and samples is sandwiched by the immobilized antibody against PAI-1 and a biotinylated polyclonal antibody against tPA, 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/tPA 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/tPA Standard:** Recombinant human PAI-1/native human tPA in a buffered protein base (8 ng, lyophilized).
- **Biotinylated tPA Antibody (100x):** A 100-fold biotinylated polyclonal antibody against human tPA (80 µl)
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 200-fold concentrate (60 µl)
- **Sample Diluent Concentrate (8x)**: A 8-fold concentrated buffered protein base (15 ml)
- **Assay Diluent:** A ready-to-use 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)
- 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 2,000 x g for 10 minutes and assay. Dilute samples 1:20 with Sample Diluent. Store samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles.
- **Tissue:** Extract tissue samples with 100 mM phosphate-buffered saline (pH7.4) containing 1% Triton X-100 and centrifuge at 14,000 x g for 20 min. Collect the supernatant and measure the protein concentration. Dilute the tissue extract 1:20 into Sample Diluent and assay. Freeze the remaining extract at -20^{0}C 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.
- **Standard Curve:** Reconstitute the 8 ng of human PAI-1/tPA Standard with 2 ml of Sample Diluent to generate a stock solution of 4 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the PAI-1/tPA standard solution (4 ng/ml) twofold with equal volume of Sample

Diluent to produce 2, 1, 0.5, 0.25, and 0.125 ng/ml. Sample Diluent serves as the zero standard (0 ng/ml).

Standard Point	Dilution	[PAI-1/tPA] (ng/ml)
P1	1 part Standard (4 ng/ml)	4.000
P2	1 part P1 + 1 part Sample Diluent	2.000
P3	1 part P2 + 1 part Sample Diluent	1.000
P4	1 part P3 + 1 part Sample Diluent	0.500
P5	1 part P4 + 1 part Sample Diluent	0.250
P6	1 part P5 + 1 part Sample Diluent	0.125
P7	Sample Diluent	0.000

- **Sample Diluent Concentrate (8x):** Dilute Sample Diluent 1:8 with reagent grade water.
- **Biotinylated tPA 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 Wash Buffer Conc. 1:10 with reagent grade water.
- **SP Conjugate (200x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:200 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 and store in a vacuum desiccator to minimize exposure to water vapor.
- Add 50 µl of standard or samples per well, and cover wells and incubate for 2 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 tPA Antibody Conjugate to each well and incubate for 30 minutes.
- Wash five times with 200 µl of Wash Buffer.
- Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes.
- Wash five times with 200 µl of Wash Buffer. Turn on the microplate reader and set up the program.
- Add 50 µl of Chromogen Substrate per well and incubate for approximately 10 minutes or till the optimal color density develop. Gently tap 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.

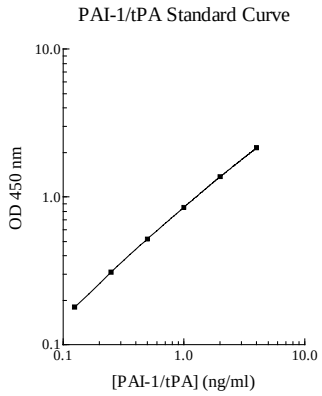
Data Analysis

- Calculate the mean value of the duplicate 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 of the linear portion of the curve using linear 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.



Performance Characteristics

- The minimum detectable level of PAI-1/tPA is typically < 50 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 6.7 % and 8.2% respectively.
- No significant cross-reactivity or interference was observed.

Reference Values

- Normal human plasma PAI-1/tPA concentration has been reported ranging approximately from 1.2 to 4.4 ng/ml (5), and from 2.4 to 8.8 ng/ml (8).

References

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