Signosis
Innovative Plate Assay Solutions

Human PAI-1 ELISA

Catalog Number EA-0207 **(For Research Use Only)**

Introduction

Plasminogen activator inhibitor 1 (PAI-1) is a member of a family of proteins that inhibit plasminogen activators. It is a single-chain glycoprotein with a molecular weight of 47 kDa. PAI-1 is the primary inhibitor of tPA and other plasminogen activators in the blood. PAI-1 is mainly produced by the endothelial cells. PAI-1 is also synthesized by adipose tissue (1). The production of PAI-1 by adipose tissue that elevates plasma PAI-1 levels was observed in insulin resistant patients. Increased PAI-1 levels have been shown to be associated with a number of atherosclerotic risk factors. Insulin and proinsulin correlate with PAI-1 levels. Patients with insulin resistance syndrome and diabetes mellitus tend to have increased PAI-1 levels. Weight loss and treatment aimed at lowering triglyceride and/or cholesterol levels have also been shown to lower PAI-1 levels.

Principle of the assay

PAI-1 ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes a mouse anti-human PAI-1 antibody for immobilization on the microtiter wells and goat anti-human PAI-1 antibodies along with streptavidin conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two antibodies, resulting in the PAI-1 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of PAI-1 is directly proportional to the color intensity of the test sample. Absorbance is measured

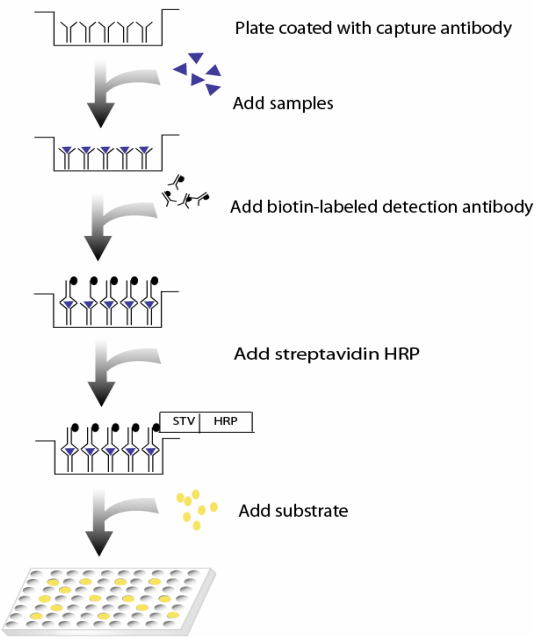


Diagram of ELISA

Materials provided with the kit

- 96 well microplate coated with a mouse anti-human PAI-1 antibody (4°C).
- Biotin labeled goat anti-human PAI-1 antibodies (-20°C).
- Streptavidin-HRP conjugate (4°C).
- Recombinant PAI-1 standard (-20°C).
- 1X Diluent buffer (4°C)
- 5X Assay wash buffer (4°C)
- Substrate (4°C)
- Stop Solution (4°C)

Material required but not provided

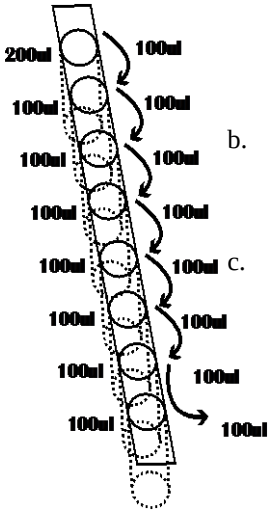
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 100 times of human recombinant PAI-1 (400ng/ml) with 1X Diluent buffer to 4000pg/ml and then 2-fold serial dilutions. To dilute 100 times of Human Recombinant PAI-1, add 2ul Human Recombinant PAI-1 in 200ul 1X Diluent Buffer (See Step 2 in "Assay Procedure" for detailed instruction)
- Use serum-free conditioned media or original or 10-fold diluted sera. Sera can be diluted with 1 X Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control.
- Dilute 400 times of biotin labeled mouse anti-human PAI-1 antibody with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

Assay procedure

1. Cut the sealing film over the plate and remove it from the desired number of well strips. Make sure the rest of wells are well sealed
2. See instruction and diagram below for standard preparation.



a. Add 200ul 1X Diluent buffer to the 1st well. Add 100ul 1X Diluent Buffer to the rest wells of strip.
b. Add appropriate amount of protein recombinant (follow instruction in "Reagent Preparation")
c. Mix dilutions in 1st well and transfer 100ul from the 1st well to the next dilution. (See picture) Incubate each well for 1 hr at room temperature with gentle shaking

3. Add 100ul of sample per well and incubate for 1 hour together with protein standard at room temperature with gentle shaking.
4. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
5. Add 100µl of diluted biotin-labeled mouse anti-human PAI-1 antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
8. Repeat the aspiration/wash as in step 4.
9. Add 100µl of substrate to each well and incubate for 10-30 minutes.
10. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
11. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

References

(1) Jenkins P. Cancer in acromegaly. Trends Endocrinology Metab 1998; 9: 360-366.

Example of standard curve