



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

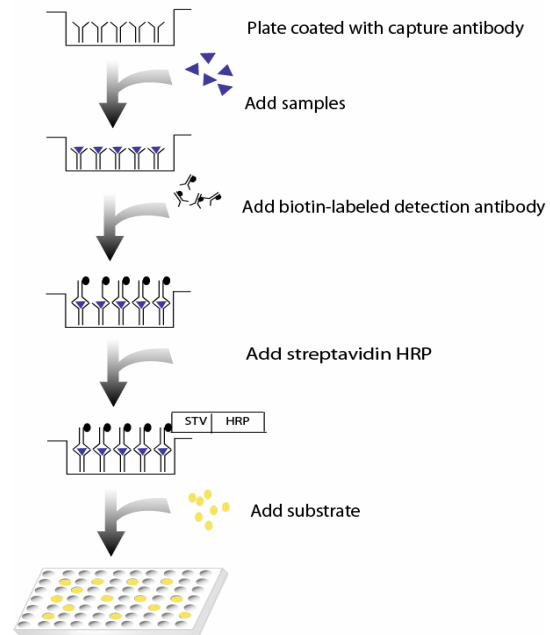


Diagram of ELISA

Materials provided with the kit

Component	Qty	Store at
96-well microplate coated with anti-human PAI-1 antibody	1	4°C
Biotin labeled anti-human PAI-1 antibody	25µL	-20°C
Recombinant Human PAI-1 standard (400ng/ml)	10µL	-20°C
Streptavidin-HRP conjugate	50µL	4°C
1x Diluent buffer	40mL	4°C
5X Assay wash buffer	40mL	4°C
Substrate	10mL	4°C
Stop solution	5mL	4°C

Material required but not provided

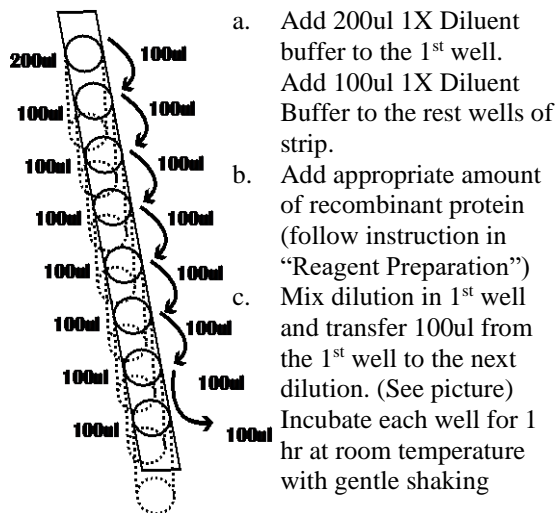
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
40ml 5x Assay wash buffer
160ml ddH₂O
- Dilute 50 times of human recombinant PAI-1 (400ng/ml) with 1X Diluent buffer to 8000pg/ml and then 2-fold serial dilutions. To dilute 50 times of Human Recombinant PAI-1, add 4ul Human Recombinant PAI-1 to 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. Calculate the number of samples to decide how many strips need to be used.
2. See instruction and diagram below for standard preparation.



3. Add 100ul of sample per well and incubate for 1-2 hours together with protein standard at room temperature with gentle shaking.
4. Aspirate each well and wash by adding 200ul of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Completely remove liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.

5. Add 100ul 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 ul 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 100ul of substrate to each well and incubate for 10-30 minutes.
10. Add 50ul 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

