

ELITEST – PAI-1 # ACK102A

Plasminogen Activator Inhibitor-1 (PAI-1) antigen

For in vitro use only

For research use only



Manufactured By: HYPHEN BioMed

INTENDED USE

ELITEST PAI-1 is an Enzyme ImmunoAssay (EIA) for the quantitative determination of Plasminogen Activator Inhibitor (PAI-1) in human plasma or cell culture supernatant.

TEST PRINCIPLE

The wells of polystyrene microplate strips have been coated with a monoclonal anti-PAI-1 (antibody to PAI-1), which constitutes the solid-phase antibody. The test sample is incubated in such well; PAI-1, if present in the sample or standard solution, will bind to the solid-phase antibody. Subsequently a monoclonal anti-PAI-1, which has been labelled with the enzyme horse-radish peroxidase (HRP), is added. With a positive reaction this labelled antibody becomes bound to any solid-phase antibody/PAI-1 complex previously formed. Incubation with enzyme substrate produces a blue colour in the test well, which turns into yellow when the reaction is stopped with sulphuric acid. The amount of colour produced in the wells is proportional to the amount of PAI-1 originally present in the sample or standard solution.

REAGENTS SUPPLIED

Each pack contains:

- 1 sachet containing a strip-holder with 12 x 8 **anti-PAI-1** (mouse monoclonal) **coated test wells** and a silicagel bag as drying agent.
- 6 vials containing 0.3 ml of **recombinant PAI-1 standard solution** at concentrations of 100, 75, 50, 25, 10 and 5 ng/ml (phosphate buffer with stabilizing proteins, EDTA, containing 0.05% Kathon CG as preservative).
- 2 vials containing 30 ml of **Sample Diluent (SD)**: phosphate buffer with stabilizing proteins, EDTA, containing 0.05% Kathon CG as preservative).
- 1 vial containing 0.3 ml of concentrated **Conjugate (C)**: mouse monoclonal anti-PAI-1 labelled with horse-radish peroxidase, containing 0.05% Kathon CG as preservative), to be diluted 100 x before use.
- 1 vial containing 30 ml of **Conjugate Diluent (CD)**: phosphate buffer with stabilizing proteins, containing 0.05% Kathon CG as preservative).
- 1 vial containing 0.5 ml of concentrated **TMB substrate solution (S)**: tetramethylbenzidine dissolved in dimethyl sulfoxide), to be diluted 100 x before use.
- 1 vial containing 30 ml of **Substrate Buffer (SB)**: phosphate citrate buffer containing 0.006% hydrogen peroxide).
- 2 vials containing 30 ml of concentrated **Wash Solution (WS)**: phosphate buffer containing 0.17% Kathon CG as preservative) to be diluted 25 x before use.
- 4 **adhesive plate sealers**.
- 1 **plastic minigrip bag** storage of unused strips.

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water.
- Sulphuric acid analytical grade in the range of 1 to 2 mol/liter (e.g. from Merck or meeting American Chemical Society Standards).
- Precision pipettes with disposable tips to deliver 20 and 200 µl.
- Optionally a multichannel pipette to deliver 20 and 200 µl can be used together with disposable V-shaped troughs for addition of conjugate, substrate and sulfuric acid.
- Timer.
- Microplate washer (alternatively, washing can be performed manually, e.g. by using a repeating syringe delivering 0.3 ml volumes and an aspirating device).
- Absorbent tissues.
- Photometric reading: microplate reader, equipped with a 450 nm filter and preferably also with 620 nm or 690 nm filter.

STORAGE AND STABILITY

- If kept at 2 to 8°C, all test reagents, including the coated test wells, are stable until the expiration date given on the pack. Do not freeze reagents.
- All reagents and the sachet containing the test wells must be brought to room temperature (15 - 30°C) approximately 30 minutes before use and must be returned to the refrigerator immediately after use.
- Unused test wells, stored at 2 - 8°C, are stable for 8 weeks if stored in the plastic minigrip bag with silicagel.

- Diluted wash solution is stable for 2 weeks, if kept at 2 - 8°C.
- Diluted conjugate is stable for 8 hours at room temperature (15 - 30°C) if kept in the dark.
- Diluted substrate is stable for 1 hour at room temperature (15 - 30°C) if kept in the dark.
- After using some of the contents of vials containing standard solutions, Sample Diluent, concentrated Conjugate, Conjugate Diluent, Concentrated Substrate, Substrate Buffer, and concentrated Wash Solution, the contents are stable until the expiration date if kept at 2 - 8°C and stored in the closed original vial.

SAFETY

All blood components and biological materials should be considered as being potentially infectious and should be handled as such. Human serum components, which are used for the reagents of this kit, have been tested by immunoassays and were found to be non-reactive for hepatitis B surface antigen, antibodies against HIV-1, HIV-2 and HCV.

Avoid contact and inhalation of TMB substrate. If substrate comes into contact with skin wash thoroughly with water.

Samples and all materials used in the assay must be considered potentially able to transmit infectious agents. They should be disposed of in accordance with established safety procedures.

SPECIMENS

- Venous blood samples are to be collected into CTAD tubes (Beckton-Dickinson, Stago) and immediately cooled on ice.
- Sample in the collection tube can be stored up to 7 hours if stored on ice (0°C).
- Before storage, plasma should be separated from blood clot or blood cells by centrifugation at 2000 to 5000 g for 20 minutes.
- Store the sample at 2 - 8°C if used within 24 hours.
- For storage up to three months, freeze in aliquots at -20°C.
- For longer storage, freeze in aliquots at -70°C.
- Repeatedly (more than 2 times) frozen and thawed samples may produce erroneous results.

PREPARATION AND MANIPULATION PROCEDURES

Preparations:

- Allow all test materials to reach room temperature (15 - 30°C) before use.
- Washing solution should be prepared by diluting concentrated Wash Solution (8) 25 x with distilled or deionized water, e.g. by diluting 24 ml to 600 ml. Prepare at least 50 ml of diluted wash solution for each test well strip.

Note: - Salt crystals may be formed in the concentrated wash solution after storage at 2 - 8°C. These crystals should be **completely** redissolved, by warming at 37°C, before dilution.

- Wash solution must be at room temperature (15 - 30°C) when used.

- Conjugate should be prepared by diluting concentrated Conjugate (4) 100 x with Conjugate Diluent (5), e.g. by diluting 20 µl to 2 ml per strip or 240 µl to 24 ml per plate.

- Substrate should be prepared by diluting concentrated TMB substrate (6) 100 x with Substrate Buffer (7), e.g. by diluting 20 µl to 2 ml per strip or 240 µl to 24 ml per plate.

Note: - Concentrated TMB should be melted completely (melting point 18°C).

Directions for washing:

Incomplete washing will adversely affect the test outcome.

Operating instructions for washing equipment should be carefully followed.

Contamination of wash solution and washer can cause extensive problems.

Therefore: - Store diluted wash solution at 2 - 8°C. Preferably use freshly prepared wash solution.

- Prerinse the washer with diluted wash solution.

- At the end of the day rinse the washer with purified water. Leave this solution in the washer until further use.

In case problems occur, disinfect wash bottles and washer overnight with 4% formaldehyde solution.

If no suitable automatic washer is available, washing can be performed manually as follows: aspirate completely the liquid from all wells by lowering an aspiration tip gently to the bottom of each well. Take care not to scratch the inside of the well surface. After the

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last aspiration fill the wells with 0.3 ml of diluted wash solution. Leave to soak for a minimum of 30 seconds, then aspirate the liquid. Perform these steps four times. After the last aspiration the washing procedure is completed by inverting the plate and tapping it dry on absorbent tissue.

Remarks and precautions:

- Do not use the kit beyond the expiration date.
- In any one test-run do not combine strip plates and conjugate from packs which have different lot numbers.
- All vessels used to prepare conjugate and substrate solutions must be cleaned thoroughly and finally rinsed with distilled water.
- Do not touch the top of the plates with your fingers to avoid contamination.
- Avoid microbial contamination of reagents.
- Ensure that the samples and standards are homogeneous before use.
- Use a new pipette-tip for each specimen aliquoted.
- To avoid contamination, do not touch the edges of the wells with the pipette tips when adding sample or conjugate.
- Do not expose substrate solution to strong light during incubation or storage. Substrate solution must be almost colourless when used.
- Solutions containing TMB, sulphuric acid or peroxide should not be in contact with metals or metal-ions, to avoid unwanted colour formation.
- Make sure no air-bubbles in the wells are present; remove any detected by tapping gently.
- If the wells can not be filled with conjugate or substrate immediately after washing, the strips may be placed upside down on a wet absorbent tissue for no longer than 15 minutes.

TEST PROCEDURE

Before starting the assay, be sure the sample diluent is at room temperature.

1. Take the strip-holder with the required number of strips, taking into account that for each test-run 6 standards and one blank should be included. Place unused strips in the plastic minigrip bag with the silicagel bag.
During the test-run the strips stay in the strip-holder and can be marked on one edge.
2. Add 200 µl of sample diluent to each test well reserved for specimen and standards. Add 20 µl of sample diluent to one test well reserved as blank.
3. Add 20 µl of the appropriate specimen or standard to each well. Make sure specimen and standards are adequately mixed with the sample diluent on a plate shaker or manually by pipetting up and down two times when specimen or standard is pipetted vertically into each well.
Note: Alternatively 1:10 dilutions of specimen and standards can be made on forehand. In that case, 200 µl of the appropriate diluted specimen, standard or blank should be added to each test well.
4. Cover the strips with an adhesive sealer.
Incubate for 60 minutes at 37°C.
Note: Prepare conjugate solution during incubation, see Preparations).
5. Wash each well 4 times (see Directions for washing).
6. Add 200 µl prepared conjugate solution to each well, tap the strip-holder carefully to mix.
7. Cover the strips with an adhesive sealer.
Incubate for 60 minutes at 37°C.
Note: Prepare substrate solution during incubation, see Preparations).
8. Wash each well 4 times (see Directions for washing).
9. Add 200 µl prepared substrate solution to each well, tap the strip-holder carefully to mix.
10. Incubate for 30 minutes at 20 - 25°C.
11. To stop the reaction add 50 µl sulphuric acid to each well, in the same sequence and at the same time intervals as the substrate solution. Tap the strip-holder carefully to ensure thorough mixing.
12. Blank the reader and read (within 15 minutes after step 11) the absorbance of the solution in the wells at 450 nm.
For dual wavelength analysis, 690 nm or 620 nm should be used as a reference wavelength.

RESULTS

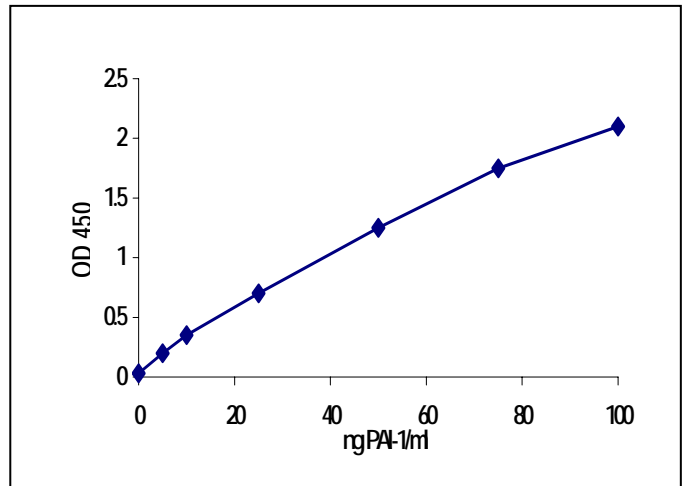
Construct the standard curve by plotting the mean absorbance values obtained for each of the PAI-1 standard solutions on the vertical (Y) axis versus the corresponding PAI-1 concentrations on the horizontal (X) axis, using rectilinear graph paper.

Draw the best fitting curve through these points.

Using the mean absorbance value for each sample to be tested, determine the corresponding concentration of PAI-1 in ng/ml from the standard curve.
If, in an initial assay, a sample is found to contain a PAI-1 concentration above 100 ng/ml, the sample can be further diluted with sample diluent. The value read from the standard curve must then be multiplied by the dilution factor.

A typical standard curve is shown below.

ELITEST PAI-1 standard curve



SUMMARY OF THE TEST PROCEDURE

Reagent	Volume	Procedure
Sample diluent (Ready to use)	200 µl	Add to one test well, reserved as blank.
	200 µl	Add to each test well, reserved for specimen and standards.
Standard or sample	20 µl	Add to each appropriate test well, mix on plate or manually by pipetting up and down, cover plate with adhesive sealer.
Incubate for 60 minutes at 37°C		
Warning : prepare conjugate before the end of incubation		
Wash solution (to be diluted 25x before use in distilled deionized water)	4 x 0.3 ml	Wash each well 4 times.
Conjugate (to be diluted 100x before use in conjugate diluent)	200 µl	Add to each well, mix gently. Cover plate with new adhesive sealer.
Incubate for 60 minutes at 37°C		
Warning : prepare substrate before the end of incubation		
Wash solution (to be diluted 25x before use in distilled or deionized water)	4 x 0.3 ml	Wash each well 4 times.
Substrate (to be diluted 100x before use in substrate buffer)	200 µl	Add to each well
Incubate for 30 minutes at 20-25°C		
Sulphuric acid	50 µl	Add to each well. Mix by tapping side of plate
Read absorbance at 450 nm within 15 minutes.		