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Human PAI-1 Total Antigen ELISA Kit

Catalog No: CKH348A **Size:** 1 x 96 wells
CKH348B **Size:** 5 x 96 wells

Introduction:

The Human PAI-1 Total Antigen ELISA is intended for the quantitative determination of total Plasminogen Activator Inhibitor 1 (PAI-1) in human plasma. This kit has been configured for research use only.

PAI-1 is involved in the regulation of the blood fibrinolytic system. Increased plasma levels of PAI-1 are implicated in the impairment of fibrinolytic function and may be associated with thrombotic diseases. Levels of PAI-1 increase with age and are elevated in conditions such as normal pregnancy, sepsis, and obesity.

Human PAI-1 present in plasma will react with the capture antibody coated and dried on a microtiter plate. Free, latent, and complexed PAI-1 will bind to the plate. Any unbound PAI-1 is washed away and an anti-PAI-1 primary antibody is added. Excess primary antibody is washed away and bound antibody, which is proportional to the total PAI-1 present in the samples, is then reacted with the secondary antibody. Following an additional washing step, TMB is then used for color development at 450 nm. The amount of color development is directly proportional to the concentration of total PAI-1 in the sample.

Reagents Included for 1 x 96 Wells:

Items	Quantity
A: Microtiter plate coated with Anti-Human PAI-1 Capture Antibody	96 wells (8 x 12-well strips)
B: Wash Buffer Concentrate (10x)	1 bottle (50 ml)
C: Human PAI-1 Standard (lyophilized)	1 vial
D: Anti-Human PAI-1 Primary pAb	1 vial
E: HRP-conjugated Anti-Rabbit IgG Secondary Antibody	1 vial
F: TMB Substrate Solution*	1 bottle (10 ml)

Avoid skin and eye contact when using TMB substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

Storage of Kit Reagents:

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary antibody may be stored at -80°C for later use. **DO NOT freeze/thaw the standards and primary antibody more than once**. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

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Materials/Reagents required but not provided:

- 1-channel pipettes covering 1-10 µl and 200-1000 µl
- 12-channel pipette for 50-500 µl
- Paper towels or Kimwipes
- 50 ml tubes
- 1 N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Buffers: TBS Buffer and 3% BSA Blocking Buffer
- Microtiter plate spectrophotometer operable at 450 nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm

Warning:

Avoid skin contact when using TMB Substrate Solution as it may be irritating to eyes, skin and respiratory system. Wear safety goggles and gloves.

Precautions:

- **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipet out of the bottle, as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** smoke, drink or eat in areas where specimens or reagents are being handled.

Preparation of Buffers, Specimen, and Standard:

TBS Buffer

0.1 M Tris + 0.15 M NaCl, pH 7.4

Wash Buffer Concentrate

Use DI water to bring to 1X

Blocking Buffer (BB)

3% BSA in TBS Buffer

Specimen Collection

Collect blood in sodium citrate or EDTA collection tubes. Immediately after collection of blood, samples must be centrifuged at 3000 x g for 15 minutes. It is important to ensure a platelet-free preparation, since platelets can release PAI-1.

The ELISA assay measures PAI-1 antigen in the 0.25-100 ng/ml range. Samples giving PAI-1 levels above 100 ng/ml must be diluted in blocking buffer or PAI-1 depleted plasma before use in the assay to bring their OD readings into the range of the standard curve.

Preparation of Standard

Reconstitute standard as directed on vial to give a 1,000 ng/mL standard solution.

Table 1: Dilution table for preparation of Human PAI-1 standard:

PAI-1 Concentration (ng/ml)	Dilutions
100	900 µl (BB) + 100 µl (1,000 ng/ml)
50	500 µl (BB) + 500 µl (100 ng/ml)
25	500 µl (BB) + 500 µl (50 ng/ml)
10	600 µl (BB) + 400 µl (25 ng/ml)
5	500 µl (BB) + 500 µl (10 ng/ml)
2	600 µl (BB) + 400 µl (5 ng/ml)
1	500 µl (BB) + 500 µl (2 ng/ml)
0.5	500 µl (BB) + 500 µl (1 ng/ml)
0.25	500 µl (BB) + 500 µl (0.5 ng/ml)
0	500 µl (BB) Zero point to determine background

NOTE: Dilutions for the standard curve must be made and applied to the plate immediately.

ELISA Method:

Be sure to read 'Preparation of Buffers, Specimen, and Standard' before carrying out the assay.

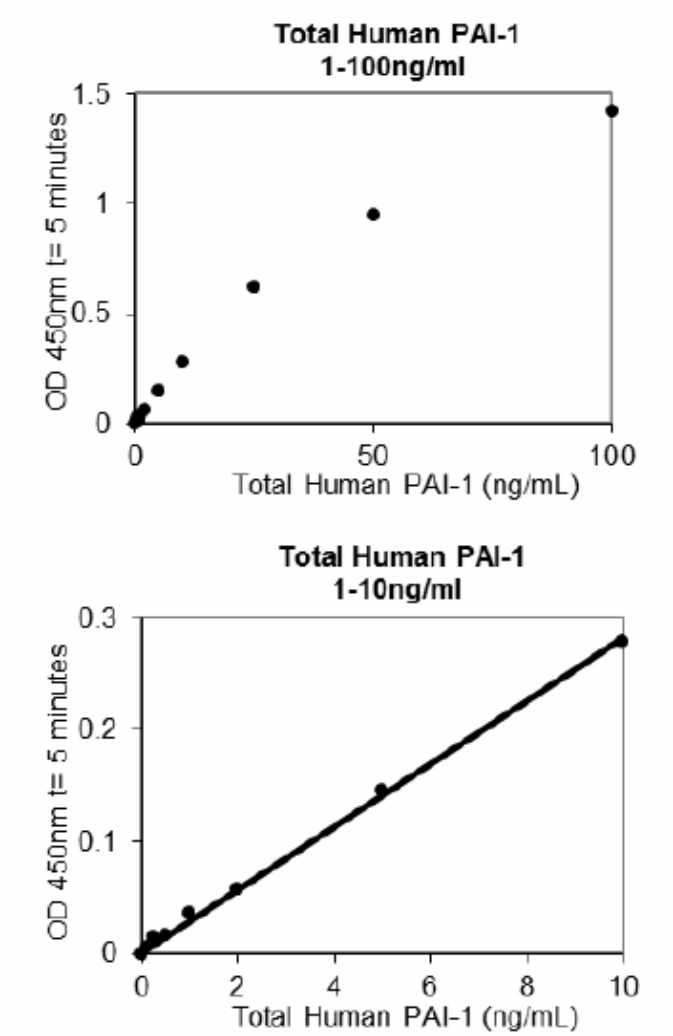
Perform assay at room temperature.

1. Remove microtiter plate from bag. Add 100 µl PAI-1 Standards in duplicate and unknowns to wells. Carefully record position of standards and unknowns.
2. Shake the plate at 300 rpm for 30 minutes.
3. Wash the wells 3 times with 300 µl Wash Buffer. Remove excess wash by gently tapping plate on paper towel or Kimwipe.
   NOTE: If the unknown is thought to have high PAI-1 levels, dilutions must be made in blocking buffer or in PAI-1 depleted plasma.
4. Reconstitute Anti-Human PAI-1 Polyclonal Primary Antibody by adding 10 ml of Blocking Buffer and agitate gently to completely dissolve contents.
5. Add 100 µl of reconstituted Anti-Human PAI-1 polyclonal Primary Antibody to all wells.
6. Shake plate at 300 rpm for 30 minutes.
7. Wash wells 3X with 300 µl Wash Buffer. Remove excess wash by gently tapping plate on paper towel or Kimwipe.
8. Dilute 1 µl HRP-conjugated Anti-Rabbit IgG Secondary Antibody in 10 ml of Blocking Buffer.
9. Add 100 µl diluted HRP-conjugated Secondary Antibody to all wells.
10. Shake plate at 300 rpm for 30 minutes.
11. Wash wells 3 times with 300 µl Wash buffer. Remove excess wash by gently tapping plate on paper towel or Kimwipe.

12. Add 100 µl of TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue.
13. Quench the reaction by adding 50 µl of 1 N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly and read final absorbance at 450 nm. For best results read plate immediately.
14. Set the absorbance at 450 nm in a microtiter plate spectrophotometer and measure the absorbance in all wells at 450 nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results:

Plot A_{450} against the amount of PAI-1 in the standards. Fit a straight line through the points using a linear fit procedure. The total PAI-1 of the unknowns can be determined from this curve.



Expected Values:

The concentration level of PAI-1 antigen in pooled normal human plasma ranged from 7.4 – 28.0 ng/ml.

Important Note: This is a generic data sheet and may be subject to change. Please see the package insert shipped with your product for current data.

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