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Human Plasminogen Activator Inhibitor type 1 Activity ELISA Kit

Catalog No: CSI19809A
CSI19809B

Size: 1 Plate (96-well)
5 Plates (5 x 96-well)

Specificity:	Human PAI-1 Activity, no reactivity with mouse and sheep; minimal with rat and porcine Vitronectin does not interfere with the detection of active PAI-1.
Sensitivity:	0.042 U/ml, minimum detectable dose (MDD)
Range:	0.125 – 100 U/ml
Sample Type:	Validated for use with citrate, EDTA, and heparin-collected plasma. (Please inquire for kit validated for culture media or tissue extracts.)

This Human Plasminogen Activator Inhibitor type 1 (PAI-1) Activity ELISA Kit is for the quantitative determination of active PAI-1 in Human plasma. 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 and sepsis.

Functionally active PAI-1 present in plasma reacts with urokinase coated and dried on a microtiter plate. Latent or complexed PAI-1 will not bind to the plate or be detected. Unbound PAI-1 samples are aspirated and an anti-human PAI-1 primary antibody is added. Excess primary antibody is then aspirated. The bound antibody, which is proportional to the original active PAI-1 present in the samples, is then reacted with the HRP-conjugated secondary antibody. Following an additional washing step, TMB substrate solution is then used for color development at 450nm. The amount of color development is directly proportional to the concentration of active PAI-1 in the sample.

One Unit of PAI-1 activity is defined as the amount of PAI- 1 that inhibits one International Unit of human single chain tPA as calibrated against the WHO International Standard for PAI-1, (NIBSC Code 92/654).
Conversion factor: 1 PAI-1 Unit = 1.34 ng

Reagents and materials supplied with the kit:

Contents	Quantity
CSI19809A-P Microtiter Strip Plate coated, blocked, and dried with uPA	1 x 96-well
CSI19809A-B Human PAI-1 Zero Unit Activity Standard, lyophilized plasma	2 vials
CSI19809A-C. Human PAI-1 High Activity Standard, lyophilized plasma	1 vial
CSI19809A-D Wash Buffer (10x)	1 bottle (50 mL)
CSI19809A-E General Assay Diluent	1 bottle (10 mL)
CSI19809A-F Anti-Human PAI-1 primary mAb, lyophilized	1 vial
CSI19809A-G HRP-conjugated anti-mouse secondary antibody	1 vial
CSI19809A-H TMB substrate solution*	1 bottle (10 mL)

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***Hazard Information:**

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. Only reconstitute one vial of 0 U standard each time the assay is performed. Reconstituted 260 U standard and primary antibody may be stored at -80°C for later use. **DO NOT freeze/thaw** the 260 U standard 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.

Materials/reagents required but not provided:

- 1-channel pipettes covering 0-10 µl and 20-200 µl
- 12-channel pipette for 50-5000 µl
- Paper towels or laboratory wipes
- Polypropylene tubes, 0.5 ml for dilution of standard
- Conical polypropylene tube, 50 ml for 1X Wah Buffer
- 1N H_2SO_4
- Sodium Chloride (NaCl)
- Deionized or distilled water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Microtiter plate spectrophotometer operable at 450 nm
- Automatic microtiter plate washer
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm

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 and avoid contact of reagents with skin.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette 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.
- The PAI-1 activity standards are of human origin. Each donor unit has been tested and found negative for the presence of HBsAg, anti-HIV 1+2, anti-HBc, and anti-HCV. Since no tests are currently available to assure that no infectious agents are present, the plasma must be treated as is recommended at Biosafety Level 2 as a potentially infectious human serum or blood specimen in the U.S. Department of Health and Human Services manual, “Biosafety in Microbiological and Biomedical Laboratories”, 5th Edition, 2009.

Preparation of Buffers, Specimen, and Standard:

TBS Buffer

0.1 M Tris, 0.15 M NaCl, pH 7.4

Blocking Buffer

3% BSA in TBS Buffer

1X Wash Buffer

Dilute 50 ml of 10x wash buffer concentrate with 450 ml deionized water.

Specimen Preparation

Collect 9 volumes of blood in 1 volume of 0.1 M trisodium citrate or acidified citrate. Immediately after collection of blood, samples must be centrifuged at 3,000 x g for 15 minutes. It is important to ensure a platelet free preparation as platelets can release PAI-1. The plasma must be transferred to a clean plastic tube and must be stored on ice prior to analysis. The PAI-1 activity samples are stable up to 24 hours or stored at -20°C for up to one month and thawed up to three times without loss of PAI-1 activity.

The assay measures active PAI-1 in the 0.125-100 U/mL range. Normal plasma should be applied directly to the plate for best results. Samples giving PAI-1 levels above 100 U/ml should be diluted in plasma devoid of active PAI-1. It is important to ensure a platelet free preparation of plasma as platelets can release PAI-1. Functionally active PAI-1 reacts with urokinase coated onto a micro titer plate.

Latent or complexed PAI-1 will not bind to the plate and will not be detected by the assay.

Preparation of Standard

Reconstitute **260 U standard** and one vial of **0 U standard** by adding 1.0 ml of DI water to each vial and agitate gently to completely dissolve contents. Make standard dilutions in 0.5 ml tubes. After making dilutions, immediately freeze 260 U standard at -80°C for later use. After reconstitution, prepare the standards according to the dilution table below.

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

PAI-1 Concentration (U/mL)	µl of 260 U/ml PAI-1 Standard	µl of 0 U/ml PAI-1 Standard	Total Volume (µl)
100	30	48	78
50	15	63	78
25	10	94	104
10	3	75	78
5	3	153	156
2	60 of **5 U/ml**	90	150
1	75 of **2 U/ml**	75	150
0.5	75 of **1 U/ml**	75	150
0.25	75 of **0.5 U/ml**	75	150
0.125	75 of **0.25 U/ml**	75	150
0	0	75	75

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.

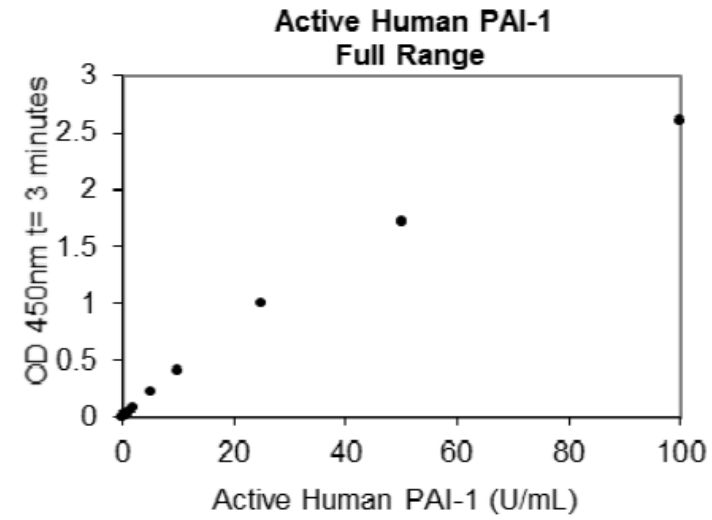
Perform assay at room temperature. Vigorously shake plate (300 rpm) at each step of the assay.

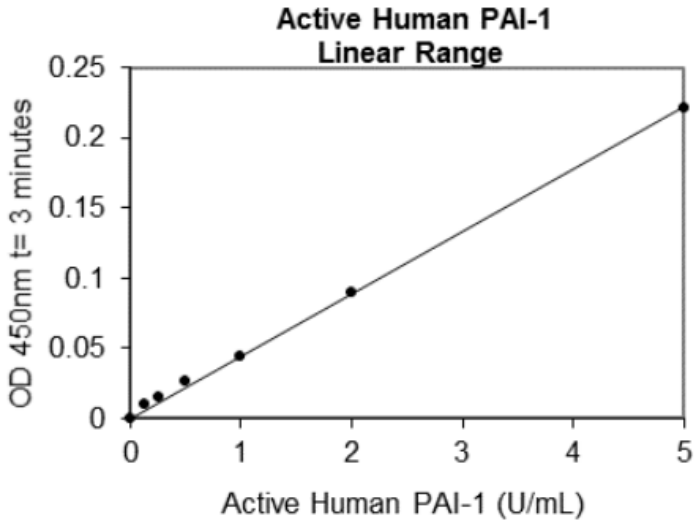
1. Remove microtiter plate from bag. Add 80 µl General Assay Diluent to wells. Add 20 µl PAI-1 standards in duplicate and unknown samples to wells. Carefully record the position of standards and unknowns.
2. Shake plate at 300 rpm for 30 minutes.
3. Wash wells 3X with 300 µl Wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipe.
   Note: If the unknown is thought to have high PAI-1 levels, dilutions may be made in plasma devoid of PAI-1 (cat # CSI19815) or in zero unit standard.
4. Add 11 mL of BSA Blocking Buffer directly to the Anti-Human PAI-1 primary antibody vial and agitate gently to completely dissolve contents.
5. Add 100 µl of reconstituted Anti-Human PAI-1 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 laboratory wipe.
8. Briefly centrifuge HRP-conjugated secondary antibody before opening. Dilute 2 µl Anti-HRP-conjugated 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 3X with 300 µl Wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipe.
12. Add 100 µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change form colorless to different shades 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 the wells in the same order as substrate upon which color will change from blue to yellow.
14. Mix thoroughly and read final absorbance values at 450 nm. For best results read plate immediately.
15. Set the absorbance at 450 nm in a microtiter plate spectrophotometer and measure the absorbance in all wells at 450 nm, A_{450}. Subtract the zero point from all standards and unknowns to determine corrected absorbance.

Calculation of Results

Plot A450 against the amount of PAI-1 in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four-parameter logistic (4PL) curve fit. The amount of PAI-1 in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):





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EXPECTED RESULTS

A study conducted in northern Sweden using 367 subjects with no pre-screening for serum triglycerides found the following normal reference range for PAI-1 (U/ml) in plasma.

	Men	Women	All
Mean	8.2 ± 6.2	7.0 ± 5.9	12.8 ± 12.1
Median	6.6	5.9	9.6
Maximum	23.3	18.0	40.3

Average levels of active PAI-1 (ng/ml) were higher in an isolated Japanese fishing village with an older population (Age= 65.6 ± 9.4)

	Men	Women	All
Mean	23.6 ± 1.4	18.1 ± 1.1	19.8 ± 1.2
N	64	122	186

A study of platelet abnormalities found that the PAI-1 concentration of normal platelet-free plasma was 21.0 ± 7.2 ng/ml (mean ± SD), platelet-rich plasma was 282.6 ± 68.0 ng/ml and serum was 270.3 ± 71.9 ng/ml. Patients with platelet abnormalities had similar PAI-1 values in PFP, PRP and serum.

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty-two zero standard replicates (range OD450: 0.057-0.067) and calculating the corresponding concentration. The MDD was 0.11 U/ml.

Intra-assay Precision: Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (U/ml)	2.80	6.82	29.6
Standard Deviation	0.257	0.323	2.57
CV (%)	9.18	4.74	8.68

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Inter-assay Precision: Three samples of known concentration were tested in ten independent assays to assess inter-assay precision.

Sample	1	2	3
n	10	10	10
Mean (U/ml)	4.20	8.07	26.8
Standard Deviation	0.399	0.763	2.10
CV (%)	9.52	9.46	7.85

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in PAI-1 depleted plasma was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (U/ml)	1.50	8.06	19.3	52.9
Average Recovery	100	101	96.7	88.1
Range	96.9 – 105%	95.0 – 106%	92.8 – 104%	83.5 – 91.5%

Linearity: To assess the linearity of the assay, pooled human plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	90.8	83.9	98.2	98.0
Range	85.4 - 95.0%	82.0 - 85.7%	92.7 - 107%	81.6 - 128%

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Specificity: This assay recognizes natural active human PAI-1. Pooled normal plasma from sheep was assayed and no significant cross-reactivity was observed. Pooled normal plasma from mouse resulted in significant color development. The following factors were prepared at 50 ng/ml in PAI-1 depleted plasma and assayed for cross-reactivity.

Recombinant mouse PAI-1	No cross-reaction
Recombinant rat PAI-1	Cross-reacts 5%
Recombinant porcine PAI-1	Cross-reacts 3%
Recombinant rabbit PAI-1	Cross-reacts 35%

Disclaimer

This information is believed to be correct but does not claim to be all-inclusive and shall only be used as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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