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Human PAI-1 Activity ELISA Kit

Catalog # HPAIKT

Strip well format. Reagents for up to 96 tests.

Rev: April 2017

INTENDED USE

This human PAI-1 activity assay is for the quantitative determination of active plasminogen activator inhibitor type 1 (PAI-1) in human plasma. **For research use only.**

BACKGROUND

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 [1,2]. Levels of PAI-1 increase with age [3] and are elevated in conditions such as normal pregnancy [4] and sepsis [5].

ASSAY PRINCIPLE

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-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 horseradish peroxidase 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.

DEFINITION OF PAI-1 UNIT

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 International Standard for PAI-1, lot 92/654 distributed by NIBSC, Holly Hill, London England.

CONVERSION FACTOR

1 PAI-1 unit = 1.34ng

REAGENTS PROVIDED

- **96-well uPA coated microtiter strip plate** (removable wells 8x12) containing uPA, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human PAI-1 zero unit activity standard:** 2 vials of lyophilized plasma
- **Human PAI-1 high activity standard:** 1 vial of lyophilized plasma
- **General Assay Diluent:** 1 bottle of 10ml
- **Anti-human PAI-1 primary antibody:** 1 vial of lyophilized monoclonal antibody
- **Anti-mouse horseradish peroxidase secondary antibody:** 1 vial of concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Only reconstitute one vial of 0 U standard each time the assay is performed. Reconstituted 450 U standard and primary antibody may be stored at -80°C for later use. Do not freeze-thaw the 195 U standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- 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.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- 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 REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

SAMPLE COLLECTION

Collect 9 volumes of blood in 1 volume of 0.1M trisodium citrate or acidified citrate. Immediately after collection of blood, samples must be centrifuged at 3000Xg 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 collected PAI-1 activity samples are stable for up to 24 hours or stored at -20°C for up to one month and thawed three times without loss of PAI-1 activity.

This kit has been validated in Citrate, EDTA, and Heparin collected plasma.

ASSAY PROCEDURE

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

Preparation of Standard

Reconstitute 450 U standard and one vial of 0 U standard by adding 1ml of DI water to each vial and agitating gently to completely dissolve contents. Prepare standard dilutions in 0.5ml tubes. After making dilutions, immediately freeze 450 U standard at -80°C for later use.

Dilution table for preparation of human PAI-1 standard:

PAI-1 concentration (U/ml)	µl of **450 U/ml** PAI-1 standard	µl of **0 U/ml** PAI-1 standard	Total volume (µl)
200	60	75	135
PAI-1 concentration (U/ml)	µl of **200 U/ml** PAI-1 intermediate	µl of **0 U/ml** PAI-1 standard	Total volume (µl)
100	40 of 200 U/ml	40	80
50	20 of 200 U/ml	60	80
25	10 of 200 U/ml	70	80
10	4 of 200 U/ml	76	80
5	4 of 200 U/ml	156	160
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 AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 80µl general assay diluent to wells. Add 20µl PAI-1 standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures active PAI-1 in the 0-100 U/ml range. If the unknown is thought to have high PAI-1 levels, dilutions should be made in plasma depleted of PAI-1 (cat# HPLA-SC-PAI) or in additional zero unit standard (HPAIKT-ZEROSTD).

Primary Antibody Addition

Reconstitute primary antibody by adding 11ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 1µl of conjugated secondary antibody in 10ml of blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl 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 by gently shaking the plate.

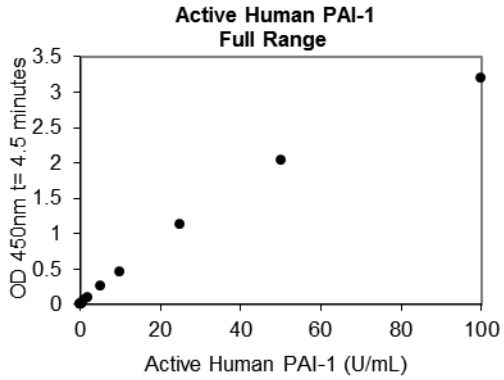
Measurement

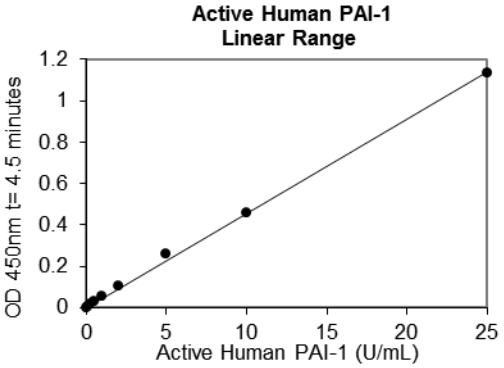
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of PAI-1 in the standards. Fit a straight line through the 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):





EXPECTED VALUES

A study conducted in Northern Sweden using 367 subjects with no pre-screening for serum triglycerides [6], observed the following normal reference range for PAI-1 (U/ml) in plasma:

	Men (20-49y)	Women (20-49y)	All (50-59y)
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) [7]:

	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 [8]. 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 OD_{450}: 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

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%

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 be used only 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.

REFERENCES

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Example of ELISA Plate Layout
96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.125 U/ml	0.25 U/ml	0.5 U/ml	1 U/ml	2 U/ml	5 U/ml	10 U/ml	25 U/ml	50 U/ml	100 U/ml	
B	0	0.125 U/ml	0.25 U/ml	0.5 U/ml	1 U/ml	2 U/ml	5 U/ml	10 U/ml	25 U/ml	50 U/ml	100 U/ml	
C												
D												
E												
F												
G												
H												