Catalog # HPAIKT

Human PAI-1 Activity Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

This human PAI-1 activity assay is for the quantitative determination of active plasminogen activator inhibitor type 1 in human plasma.

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 [5] and are elevated in conditions such as normal pregnancy [3] and sepsis [4].

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 tPA, lot 92/654 distributed by NIBSC, Holly Hill, London England.

REAGENTS PROVIDED

- **uPA coated plate:**
  1-96 well immulon microtiter plate (8X12 well removable strips) coated with uPA, blocked and dried
- **Human PAI-1 activity standard, 0 U/ml:**
  2 vials of 1.0ml lyophilized plasma with 0 U/ml PAI-1
- **Human PAI-1 activity standard, 220 U/ml:**
  1 vial of 1.0ml lyophilized plasma with lot specific PAI-1 activity
- **10X Wash Buffer:**
  1 bottle of 50ml wash; bring to 1X using DI water
- **General Assay Diluent:**
  1 bottle of 10ml diluent
- **Anti-human PAI-1 primary antibody:**
  1 vial of lyophilized monoclonal anti-human PAI-1 antibody
- **Horseradish peroxidase secondary:**
  1 vial of concentrated HRP labeled secondary antibody
- **TMB substrate solution:**
  1 bottle of 10 ml solution

STORAGE AND STABILITY

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 may be stored at -70°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.

REAGENTS AND EQUIPMENT REQUIRED

- 1-channel pipettes covering 0-10 and 20-200µl
- 12-channel pipette for 500-5000µl
- Paper towels or kimwipes
- 50ml tubes
- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm
- 0.5ml microcentrifuge tubes
- TBS buffer
- 3% Blocking buffer

WARNINGS

Warning - The PAI-1 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 the Biosafety Level 2 as potentially infectious human serum or blood specimen in the Centres for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories", 1984.
Warning – Avoid skin and eye contact when using TMB One substrate solution since 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 pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- All kit components must be kept refrigerated (4°C).
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.10M TRIS, 0.15M NaCl, pH 7.4
- **Blocking buffer (BSA):** 3% BSA in TBS buffer

SPECIMEN COLLECTION

Collect 9 volumes of blood in 1 volume of 0.1M trisodium citrate or acidified citrate, preferably using Stabilyte ™ evacuated vials (Biopool, cat# 102080). 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 PAI-1 activity samples collected in the Stabilyte media 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.

ASSAY PROCEDURE

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

Preparation of Standard:
Prepare the PAI-1 standard according to the dilution table insert found in the kit. Reconstitute each standard (0 and high U/ml) using 1ml of DI water for each. Make standard dilutions in 0.5ml microcentrifuge tubes.

NOTE: DILUTIONS FOR THE STANDARD CURVE MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.
Reconstitute standards as directed on each vial.

PAI-1 concentration (U/ml)	µl of **"220 U/ml"** PAI-1 standard	µl of **"0 U/ml"** PAI-1 standard	**Total volume(µl)**
100	45	54	99
50	25	85	110
25	10	78	88
10	5	105	110
5	3	129	132
2	3	327	330
1	100 of **2**	100	200
0.5	100 of **1**	100	200
0.25	100 of **0.5**	100	200
0.125	100 of **0.25**	100	200
0	0	100	100

Standard and Unknown Addition:
Remove microtiter plate from bag and add 80µl general assay diluent and 20µl PAI-1 standards (enough for duplicates) 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: If the unknown is thought to have high PAI-1 levels, dilutions may be made in plasma devoid of PAI-1.

Primary Antibody Addition:
Add 11ml BSA blocking buffer directly to the primary antibody 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.

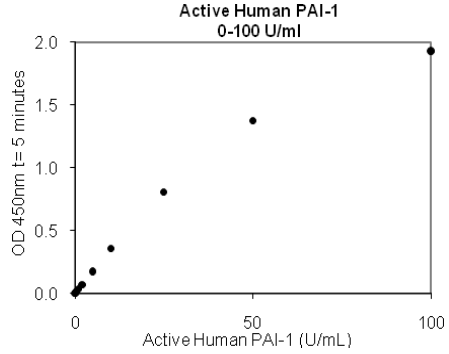
Horseradish peroxidase conjugate Secondary Antibody Addition:
Dilute 1µl conjugated secondary antibody in 10ml BSA blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by tapping plate on paper towel or kimwipe.

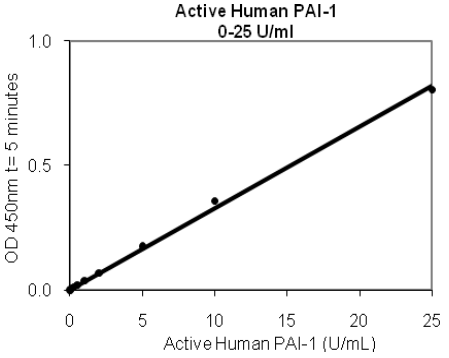
Substrate Incubation:
Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly and read final absorbance values at 450nm. For best results read plate immediately.

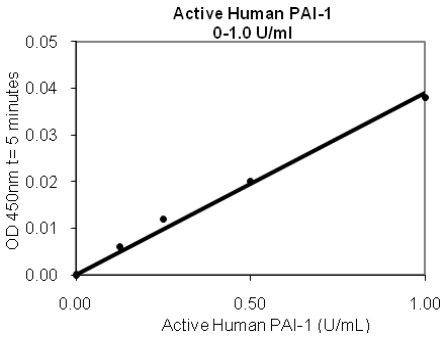
Measurement:
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm, A_{450}.

Assay Calibration:
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 PAI-1 activity in the unknowns can be determined from this curve.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)







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.

CONVERSION FACTOR

1 PAI-1 unit = 1.34ng

PERFORMANCE CHARACTERISTICS

- The assay measures active PAI-1 in the 0-100 U/ml range. Samples giving PAI-1 levels above 100 U/ml should be diluted in plasma devoid of active PAI-1.
- This kit has also been validated in Citrate, EDTA, and Heparin collected plasma.
- In house testing has determined that Vitronectin does not interfere with the detection of active PAI-1.
- This kit does not cross react with active mouse PAI-1 in plasma.

Linearity slope = 0.9183
Correlation coefficient = 0.9964

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 or from contact with the above product.

REFERENCES

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4. Colucci, M.: Generation in plasma of a fast acting inhibitor plasminogen activator in response to endotoxin stimulation. J. Clin. Invest., **75**:818-824,1985.

5. Kruithof, EK: Plasminogen activator inhibitor 1: development of a radioimmunoassay and observations on its plasma concentration during venous occlusion and after platelet aggregation. Blood, **70**:1645-1653, 1987.
6. Rånby, M.: Activity of plasminogen activator inhibitor type 1 (PAI-1) in a population of Northern Sweden. Fibrinolysis, **4**:54-55, 1990.
7. Enomoto, M *et al.*: Positive association of serum levels of advanced glycation end products with thrombogenic markers in humans. Metabolism, **55**: 912-917, 2006.
8. Booth, NA *et al.*: Plasminogen activator inhibitor (PAI-1) in plasma and platelets. Br J Haematol, **70**: 327-333, 1988.

Example of Plate Layout
Standards: 22 wells Samples: 74 wells

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