Version 03-05/24

User's Manual

Active Rat PAI-1 ELISA Kit

REF **DEIASL030**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

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

General Description

Plasminogen activator inhibitor-1 (PAI-1) is a serine protease inhibitor that is an important regulator of fibrinolysis and extracellular matrix turnover [1,2,6]. PAI-1 may be important in hepatocyte growth and proliferation in vivo. Increased PAI-1 levels may increase the risk for myocardial infarction, atherosclerosis, and retenosis [3,4]. Increased PAI-1 levels may also play an important role of the development and pathogenesis of diabetic nephropathy [5]. Decreased levels may reduce thrombotic events [7].

Principles of Testing

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 and will not be detected. Unbound PAI-1 samples are 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 original active PAI-1 present in the samples, is then reacted with the horseradish peroxidase secondary antibody. Following an additional washing step, TMB 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.

Reagents And Materials Provided

1. 96-well uPA coated microtiter strip plate (removable wells 8x12) containing uPA, blocked and dried.
2. 10X Wash Buffer: 1 bottle of 50mL.
3. Rat PAI-1 activity standard: 1 vial lyophilized standard.
4. Anti-rat PAI-1 primary antibody: 1 vial lyophilized monoclonal antibody.
5. Anti-rabbit horseradish peroxidase secondary antibody: 1 vial concentrated HRP labeled antibody.
6. TMB substrate solution: 1 bottle of 10mL solution.

Materials Required But Not Supplied

1. Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement.
2. Manifold dispenser/aspirator or automated microplate washer.
3. Microplate reader capable of measuring absorbance at 450 nm.
4. Pipettes and Pipette tips.
5. Deionized or distilled water.
6. Polypropylene tubes for dilution of standard.

7. Paper towels or laboratory wipes.
8. 1N H2SO4 or 1N HCl
9. Bovine Serum Albumin Fraction V (BSA)
10. Tris (hydroxymenthyl) aminomethane (Tris)
11. Sodium Chloride (NaCl)

Storage

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard and primary may be stored at -80°C for later use. Do not freeze-thaw the 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.

Specimen Collection And Preparation

Collect 9 volumes of blood in 1 volume of a 3.8% 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 since platelets can release PAI-1 [4]. The plasma must be stored on ice prior to analysis. The PAI-1 activity samples collected is 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.

Reagent Preparation

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

Assay Procedure

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

Preparation of Standard

Reconstitute standard by adding 5mL of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 50ng/mL standard solution.

Dilution table for preparation of rat PAI-1 standard:

PAI-1 concentration (ng/mL)	Dilutions
50	100µL from standard vial
25	500µL (BB) + 500µL (50ng/mL)
10	600µL (BB) + 400µL (25ng/mL)
5	500µL (BB) + 500µL (10ng/mL)
2	600µL (BB) + 400µL (5ng/mL)
1	500µL (BB) + 500µL (2ng/mL)
0.5	500µL (BB) + 500µL (1ng/mL)
0.25	500µL (BB) + 500µL (0.5ng/mL)
0.1	600µL (BB) + 400µL (0.25ng/mL)
0	500µL (BB) Zero point to determine background

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 100µL 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 0.1-50 ng/mL range. Plasma and serum samples should be applied directly to the plate without dilution.

Primary Antibody Addition

Reconstitute primary antibody by adding 10mL of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µL to all wells. Shake plate at 300 rpm 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. For best results read plate immediately. Subtract zero point from all standards and unknowns to determine corrected absorbance (A450).

Calculation

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

Interpretation Of Results

The concentration level of PAI-1 in rat plasma was found to be 1.0 ± 0.5 ng/mL for active PAI-1 and 1.8 ± 0.9 ng/mL for PAI-1 antigen [8].

Abnormalities in PAI-1 levels have been reported in the following conditions:

(1) Artherosclerosis: Increased PAI-1 levels may contribute to artherosclerosis [3,4].

(2) Diabetes: Elevated PAI-1 levels in rats may contribute to the development and pathogenesis of diabetic nephropathy [5].

(3) Myocardial Infarction: Increased PAI-1 levels may contribute to myocardial infarction [3,8].

(4) Restenosis: Increased PAI-1 levels is associated with restenosis [3].

(5) Thrombosis: Decreased PAI-1 levels may reducethrombotic events [7].

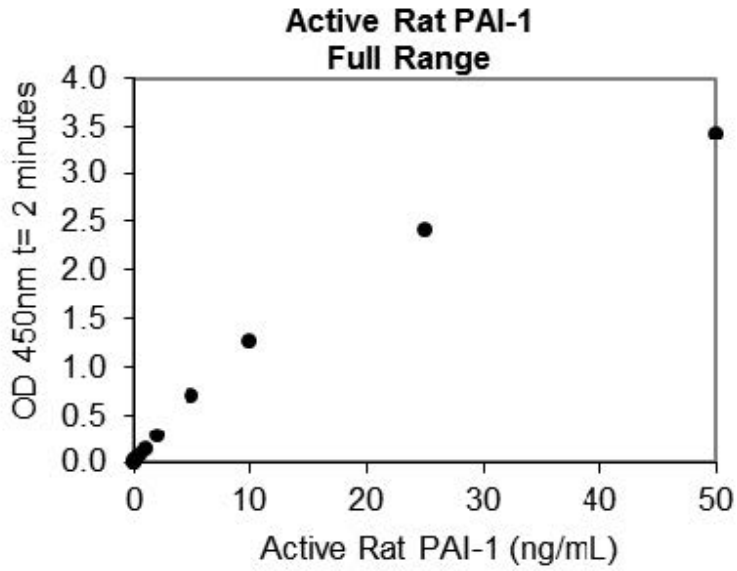
(6) Deep Venous Thrombosis: Elevated PAI-1 levels may be associated with deep venous thrombosis [8].

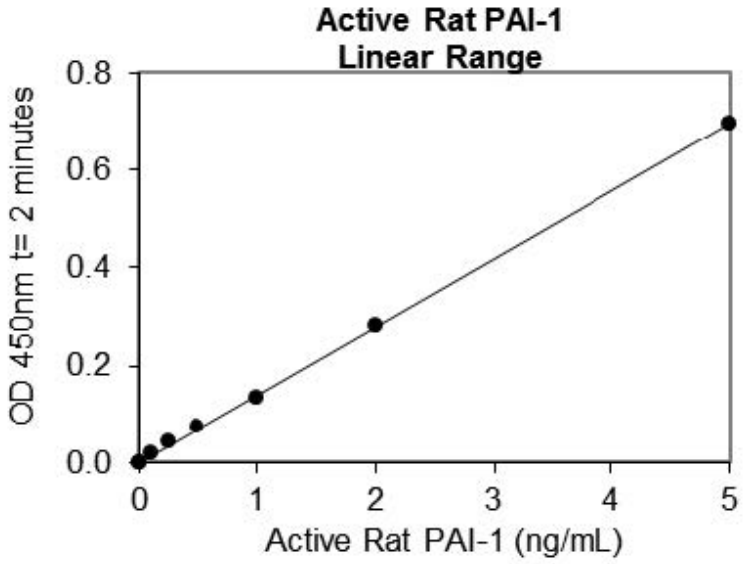
(7) Coronary Artery Disease: Elevated PAI-1 levels may increase the risk of coronary artery disease [8].

(8) Endotoxemia: Endotoxin induces a large increase in PAI-1 levels (100- to 200-fold) [8].

Typical Standard Curve

A typical standard curve (EXAMPLE ONLY):





Reference Values

Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean (ng/mL)
Citrate Plasma	Undiluted	1.4-2.8

Sensitivity

The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD450: 0.064-0.071) and calculating the corresponding concentration. The MDD was 0.032ng/mL.

Specificity

This assay recognizes natural and recombinant active rat PAI-1. Pooled normal plasma from mouse and dog was assayed and significant crossreactivity was observed. Pooled normal plasma from human and pig was assayed and minor cross-reactivity was observed. Pooled normal plasma from sheep was assayed and no significant cross-reactivity was observed. Pooled normal plasma from rabbit resulted in significant color development.

Linearity

To assess the linearity of the assay, pooled citrated rat plasma samples were serially diluted to produce samples with values within the dynamic range of the assay. Plasma samples did not demonstrate linearity and should be analyzed without dilution.

Blocking buffer 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	103	104	104	112
Range	97-112%	102-107%	100-107%	100-120%

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 (ng/mL)	0.721	2.24	1.13	21.5
Average % Recovery	103	102	102	108
Range	97-109%	96-105%	98-105%	97-112%

Reproducibility

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

Sample	1	2	3
n	20	20	20
Mean (ng/mL)	0.957	2.25	15.6
Standard Deviation	0.088	0.134	1.01
CV (%)	9.24	5.97	6.48

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 (ng/mL)	0.926	1.40	9.17
Standard Deviation	0.113	0.096	0.858
CV (%)	12.2	6.86	9.36

Precautions

1. FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
2. 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.
3. Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
4. Keep plate covered except when adding reagents, washing, or reading.
5. DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
6. DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

References

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