Version 27-04/24

User's Manual

Rat PAI-1 Total Antigen ELISA Kit

REF **DEIASL033**

Σ **96T**

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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) total antigen assay is intended for the quantitative determination of total PAI-1 in biological fluids. For research use only.

General Description

Plasminogen activator inhibitor-1 (PAI-1) is a central regulator of the blood fibrinolytic system [1]. Clinical studies have indicated that increased PAI-1 levels increase the risk for thrombosis, whereas decreased levels may cause recurrent bleeding [2].

Principles of Testing

Rat PAI-1 present in plasma reacts with the capture antibody coated and dried on a microtiter plate. Free, latent, and complexed PAI-1 will bind to the plate. After appropriate washing steps, biotin labeled primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with horseradish peroxidase conjugated streptavidin. TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of rat PAI-1. The amount of color development is directly proportional to the concentration of total PAI-1 in the sample.

Reagents And Materials Provided

1. 96-well antibody coated microtiter strip plate (removable wells 8x12) containing uPA, blocked and dried.
2. 10X Wash Buffer: 1 bottle of 50mL.
3. Rat PAI-1 standard: 1 vial lyophilized standard.
4. Anti-rat PAI-1 primary antibody: 1 vial lyophilized monoclonal antibody.
5. Horseradish peroxidase-conjugated streptavidin: 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 H_2SO_4 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 plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples of rat serum, urine, cell culture media, or tissue extracts may also be used.

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)
0.05	500µL (BB) + 500µL (0.1ng/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 0.05-50 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 2.5µL of HRP-conjugated secondary antibody into 2.5mL of blocking buffer to generate a 1;1,000 dilution. Add 0.2 mL of 1:1,0000 dilution to 9.8mL of blocking buffer to generate 1:50,000 dilution. Add 100µL 1:50,000 dilution 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 2-5 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 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.

Interpretation Of Results

The level of PAI-1 antigen in rat plasma was 1.8 ± 0.9 ng/ml (mean ± SD, n=18), with a corresponding value of 1.0 ± 0.5 ng/ml for PAI-1 activity [3].

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

(1) Endotoxemia: Endotoxin induces a large increase in PAI-1 levels (100-200 fold) [3].

(2) Hyperglycemia, hyperinsulinemia, and insulin resistance: Elevated PAI-1 levels in obese and diabetic mice contribute to these metabolic disorders [4,5].

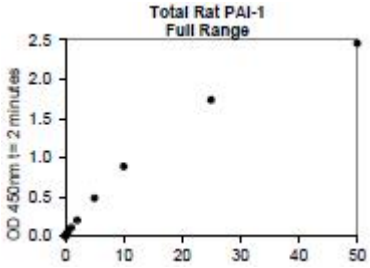
(3) Vascular thrombosis: Increased PAI-1 levels may contribute to venous thrombosis [1].

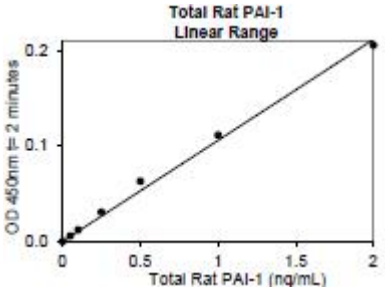
(4) Myocardial Infarction: Increased PAI-1 levels may contribute to myocardial infarction [1].

(5)Cirrhosis: Cirrhotic rat liver expressed an increased level of PAI-1 compared to normal liver [6].

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	2.27
	1:2	2.33
	1:4	2.32
	1:8	2.33

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: 0.123-0.132) and calculating the corresponding concentration. The MDD was 0.047ng/mL.

Specificity

These studies are currently in progress. Please contact us for more information.

Linearity

These studies are currently in progress. Please contact us for more information.

Recovery

These studies are currently in progress. Please contact us for more information.

Reproducibility

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

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

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

1. Eitzman DT, et al.: Blood. 2000, 95(2): 577-580.
2. Kawasaki T, et al.: Blood. 2000, 96(1): 153-160.
3. Ngo TH, et al.: Thromb Haemostas. 1998, 79(4): 808-812.
4. Schafer K, et al.: FASEB J. 2001, 15: 1840-1842.
5. Samad F, et al.: PNAS. 1996, 96(12): 6902-6907.
6. Seki T, et al.: Thromb Haemostas. 1996, 75(5): 801-807.