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Rat PAI-1 Total Antigen ELISA Kit

Catalog # RPAIKT-TOT

Strip well format. Reagents for up to 96 tests.

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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.

BACKGROUND

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].

ASSAY PRINCIPLE

Rat PAI-1 present in samples reacts with the capture antibody coated on the 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 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 PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-rat PAI-1 antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Rat PAI-1 standard:** 1 vial lyophilized standard
- **Anti-rat PAI-1 primary antibody:** 1 vial lyophilized monoclonal antibody
- **Horseradish peroxidase-conjugated streptavidin:** 1 vial concentrated HRP labeled streptavidin
- **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. Reconstituted standards 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.

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.

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 plasma using EDTA 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.

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 of 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 total PAI-1 in the 0.05-50 ng/ml range. A 1:2-1:10 dilution for normal rat plasma is suggested for best results. If the unknown is thought to have high PAI-1 levels, dilutions may be made in plasma devoid of PAI-1 (Cat# RPLA-SC-PAI) or in blocking buffer.

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 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Streptavidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 2.5µl of HRP conjugated streptavidin into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.2ml of 1:1,000 dilution to 9.8ml of blocking buffer to generate a 1:50,000 dilution. Add 100µl of the 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.

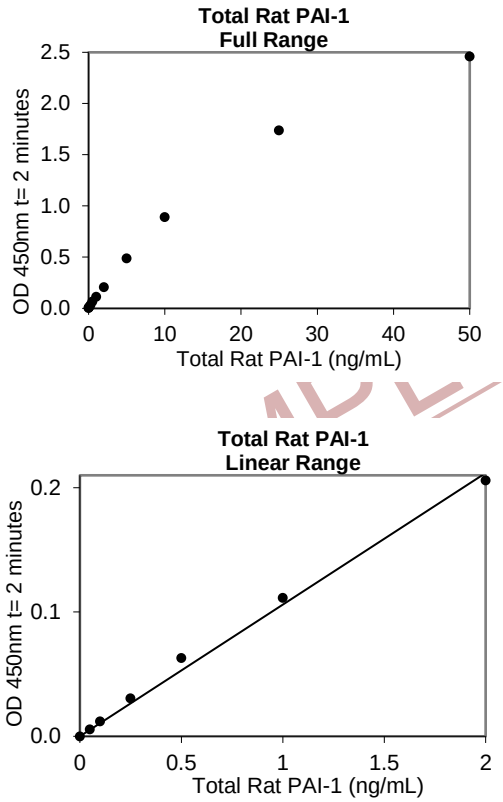
Measurement

Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. 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

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:

- Endotoxemia: Endotoxin induces a large increase in PAI-1 levels (100-200 fold) [3].
- Hyperglycemia, hyperinsulinemia, and insulin resistance: Elevated PAI-1 levels in obese and diabetic mice contribute to these metabolic disorders [4,5].
- Vascular thrombosis: Increased PAI-1 levels may contribute to venous thrombosis [1].
- Myocardial Infarction: Increased PAI-1 levels may contribute to myocardial infarction [1].
- Cirrhosis: Cirrhotic rat liver expressed an increased level of PAI-1 compared to normal liver [6].

PERFORMANCE CHARACTERISTICS

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 OD_{450}: 0.123-0.132) and calculating the corresponding concentration. The MDD was 0.047ng/ml.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: 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.

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

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

Sample Values: Samples were evaluated for the presence of the antigen.

Sample Type	Dilution	Mean (ng/ml)
Citrate Plasma	Undiluted	2.27
	1:2	2.33
	1:4	2.32
	1:8	2.23

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

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.

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	0.05 ng/ml	0.1 ng/ml	0.25 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml	
B	0	0.05 ng/ml	0.1 ng/ml	0.25 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml	
C												
D												
E												
F												
G												
H												