Catalog # IRPAIKT-TOT

Rat PAI-1 Total Antigen Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

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.

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

Pat PAI-1 will bind to the capture antibody coated on the microtiter plate. Free, latent, and complexed PAI-1 will will react with the capture antibody on the plate. Any unbound PAI-1 is 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 total PAI-1 present in the samples, is then reacted with a 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 total PAI-1 in the sample.

REAGENTS PROVIDED

- ♦Immunoassay plate:
  1-96 well immulon plate coated with anti rat PAI-1 capture antibody, blocked, and dried
- ♦10X Wash Buffer:
  1 bottle of 50ml wash; bring to 1X using DI water
- ♦Rat PAI-1 activity standard:
  1 vial lyophilized standard
- ♦Anti-rat PAI-1 primary antibody:
  1 vial lyophilized polyclonal anti-rat antibody
- ♦Anti-rabbit horseradish peroxidase secondary antibody:
  1 vial concentrated HRP labeled antibody
- ♦TMB substrate solution:
  1 bottle 10ml 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 0-10µl and 200-1000µl
- •12-channel pipette covering 50-500µl
- •Paper towels or kimwipes
- •50ml tubes
- •1N H_2SO_4
- •DI water
- •Magnetic stirrer and stir-bars

- Plastic containers with lids
- TBS buffer
- 3% Blocking buffer
- Microtiter plate spectrophotometer operable at 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

WARNINGS

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.
- **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:** 3% BSA in TBS buffer

SPECIMEN COLLECTION

Samples of rat plasma, serum, urine, cell culture media, or tissue extracts may be applied directly to the plate.

The assay measures total PAI-1 in the 0.05-50 ng/ml range. Samples giving
PAI-1 levels above 50ng/ml should be diluted in plasma or similar fluid devoid of PAI-1.

ASSAY PROCEDURE

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

Preparation of Standard:

Reconstitute standard as directed on vial and agitate gently to completely dissolve contents. Prepare dilutions of PAI-1 standard according to the dilution table

Reconstitute standard as directed on vial to give a 50ng/ml standard solution.

PAI concentration (ng/ml)	Dilutions
50	100µl from standard vial
25	500µl (BSA) + 500µl (50ng/ml)
10	600µl (BSA) + 400µl (25ng/ml)
5	500µl (BSA) + 500µl (10ng/ml)
2	600µl (BSA) + 400µl (5ng/ml)
1	500µl (BSA) + 500µl (2ng/ml)
0.5	500µl (BSA) + 500µl (1ng/ml)
0.25	500µl (BSA) + 500µl (0.5ng/ml)
0.1	600µl (BSA) + 400µl (0.25ng/ml)
0.05	500µl (BSA) + 500µl (0.1ng/ml)

NOTE: DILUTIONS FOR THE STANDARD CURVE 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: If the unknown is thought to have high PAI-1 levels, dilutions may be made in plasma devoid of PAI-1, or in blocking buffer.

Primary Antibody Addition:

Reconstitute primary antibody as directed on 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:

Dilute 1µl conjugated secondary antibody in 10ml of 3% 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 gently tapping plate on paper towel or kimwipe.

Substrate Incubation:

Add 100µl of substrate solution to all wells and shake plate at 300rpm for
2-10 minutes. Quench the reaction with the addition of 50µl of 1N H_2SO_4 and read final absorbance values at 450nm.
NOTE: Time for substrate development is dependent on needs of researcher.

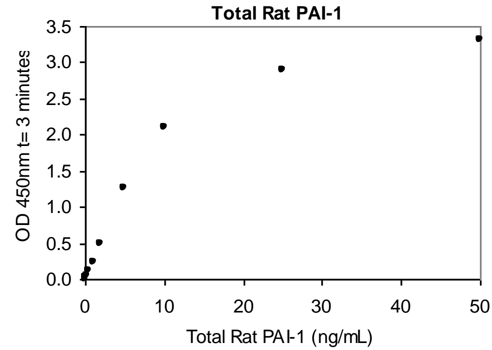
Measurement:

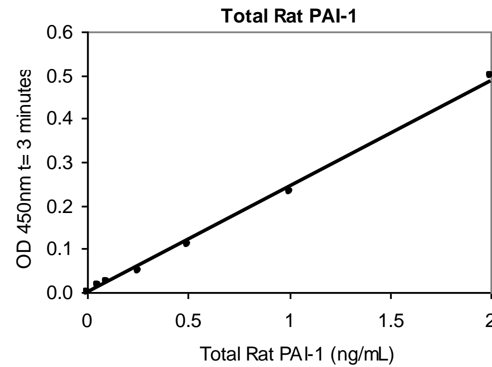
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm, A_{450}.

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

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 condition:

- ♦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 [6].
- ♦Myocardial Infarction: Increased PAI-1 levels may contribute to myocardial infarction [6].

- Cirrhosis: Cirrhotic rat liver expressed an increased level of PAI-1 compared to normal liver [7].

PERFORMANCE CHARACTERISTICS

Sensitivity = 0.032 ng/ml
(calculated by determining the OD of 20 reps of So and 20 reps of the low standard)
Linearity
The slope = 1.0813
Correlation coefficient = 0.9865

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

1. Eitzman DT, *et al*.: Plasminogen activator inhibitor-1 and vitronectin promote vascular thrombosis in mice. Blood, **95(2)**: 577-580, 2000.
2. Kawasaki T, *et al*.: Vascular release of plasminogen activator inhibitor-1 impairs fibrinolysis during acute arterial thrombosis in mice. Blood, **96(1)**: 153-160, 2000.
3. Ngo TH, *et al*.: Monoclonal antibody-based immunoassays for the specific quantitation of rat PAI-1 antigen and activity in biological samples.. Thromb Haemostas., **79(4**): 808-812, 1998.
4. Schafer K, *et al*.: Disruption of the plasminogen activator inhibitor 1 gene reduces the adiposity and improves the metabolic profile of genetically obese and diabetic ob/ob mice. FASEB J, **15**: 1840-1842, 2001.
5. Samad F, *et al*.: Tumor necrosis factor alpha is a key component in the obesity-linked elevation of plasminogen activator inhibitor 1. Proc Natl Acad Sci USA, **96**(12): 6902-6907, 1996.
6. Eitzman, DT, *et al*.: Plasminogen activator inhibitor-1 and vitronectin promote vascular thrombosis in mice. Blood, **95**(2): 577-580, 2000.
7. Seki T, *et al*.: Production of tissue-type plasminogen activator (t-PA) and type-1 plasminogen activator inhibitor (PAI-1) in mildly cirrhotic rat liver. Thromb Haemostas., **75(5**): 801-807, 1996.