Rat PAI-1 Activity Assay

Catalog No. IRPAIKT

Lot No. 512

Intended Use

RatPAI-1 activity assay is intended for the quantitative determination of active plasminogen activator inhibitor type 1 in rat plasma.

Background

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].

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 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 Provided

uPA coated plate:
1-96 well immulon strip plate (removable 8X12 wells) coated, blocked, and dried with uPA

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-mouse antibody

Anti-rabbit horseradish peroxidase secondary antibody:
1 vial concentrated HRP labeled antibody

TMB substrate solution:
1 bottle of 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.

Required Reagents and Equipment

1-channel pipettes covering 0-10ul and 200-1000ul
12-channel pipette covering 30-300ul
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 and Precautions

Warning – Avoid skin and eye contact when using TMB substrate solution. It may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

- 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.

Preperation of Reagents

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

Specimen Collection

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 anaylsis. 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.

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 the PAI-1 standard according to the dilution table

Dilution table for preparation of mouse IgG standards:

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)*
0	*500µl (BSA)Zero point to determine background*

Standard and Unknown Addition:

Remove microtiter plate from bag. Add 100 l PAI-1 standards (enough for duplicates) and unknowns to wells. Carefully record the position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300ul 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 3% BSA blocking buffer.

Primary Antibody Addition:

Reconstitute primary antibody as directed on vial and agitate gently to completely dissolve contents. Add 100 l to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 l wash buffer. Remove excess

Assay Procedure Continued

Secondary Antibody Addition:

Dilute 1µl of the conjugated secondary antibody into 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 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 H2SO4 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

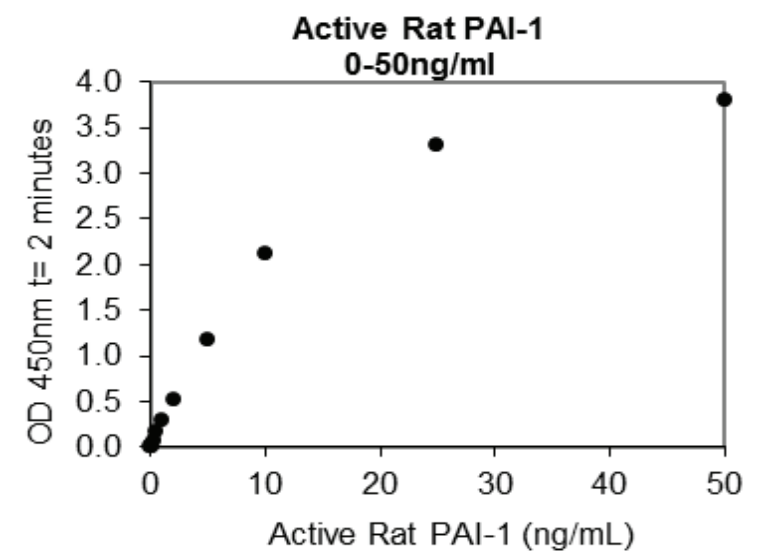
Measurement:

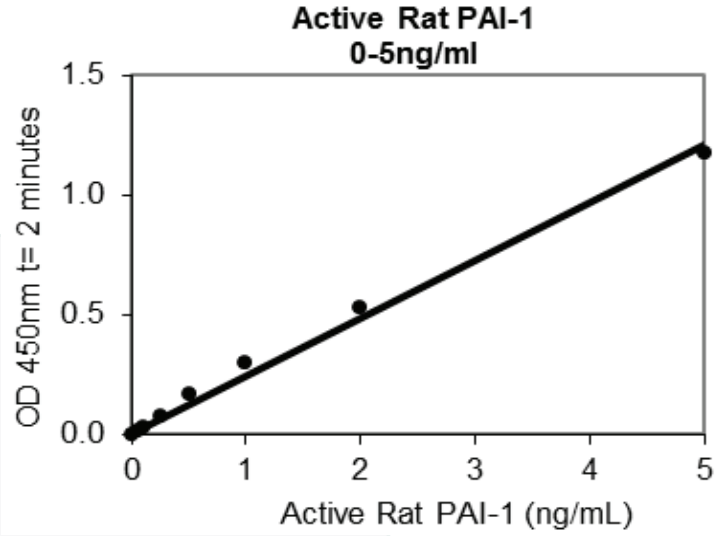
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A450).

Assay Calibration:

Plot A450 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.

Standard Curve Examples





Expected Values

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

- Artherosclerosis: Increased PAI-1 levels may contribute to artherosclerosis [3,4].
- Diabetes: Elevated PAI-1 levels in rats may contribute to the development and pathogenesis of diabetic nephropathy [5].
- Myocardial Infarction: Increased PAI-1 levels may contribute to myocardial infarction [3,8].
- Restenosis: Increased PAI-1 levels is associated with restenosis [3].
- Thrombosis: Decreased PAI-1 levels may reduce thrombotic events [7].
- Deep Venous Thrombosis: Elevated PAI-1 levels may be associated with deep venous thrombosis [8].
- Coronary Artery Disease: Elevated PAI-1 levels may increase the risk of coronary artery disease [8].
- Endotoxemia: Endotoxin induces a large increase in PAI-1 levels (100- to 200-fold) [8].

Performance Characteristics

Sensitivity = 0.011 ng/ml

(calculated by determining the OD of 20 reps of So and 20 reps of the low standard)

Linearity

The slope = 0.9607

Correlation coefficient = 0.9997

Intra Assay Precision

High 3.5%, Medium 4.9%, Low 2.8%

(calculated by running 24 reps of each concentration in an assay)

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.