

Rat Plasminogen Activator Inhibitor 1 Activity ELISA Kit

Catalog No: CSI19918A
CSI19918B

Size: 1 x 96 wells
5 x 96 wells

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 retinosis [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-rat antibody

◆ **Anti-rabbit horseradish peroxidase secondary antibody:**

1 vial concentrated HRP labeled antibody

◆ **TMB substrate solution:**

1 bottle of 10ml solution



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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 30-300µl
- Paper towels or kimwipes
- 50ml tubes
- 1N H₂SO₄
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- TBS buffer
- 3% Blocking buffer
- Microtiter plate spectrophotometer operable at 405nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

Warnings:

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 (BSA):** 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 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.

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 to give a 50ng/ml standard solution.

PAI-1 concentration (ng/ml)	Dilutions
50	100µl from standard vial
25	500µl (BSA)+ 500µl (50 ng/ml)
10	600µl (BSA) + 400µl (25 ng/ml)
5	500µl (BSA) + 500µl (10 ng/ml)
2	600µl (BSA) + 400µl (5 ng/ml)
1	500µl (BSA)+ 500µl (2 ng/ml)
0.5	500µl (BSA) + 500µl (1ng/ml)
0.25	500µl (BSA)+ 500µl (0.5 ng/ml)
0.1	600µl (BSA) + 400µl (0.25 ng/ml)
0.05	500µl (BSA) + 500µl (0.1 ng/ml)
0	500µl (BSA)

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. Add 100µl 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 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 3% BSA blocking buffer.



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Primary Antibody Addition:

Reconstitute primary antibody as directed on vial and mix gently to completely dissolve contents. Add 100 μ l to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μ l wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Secondary Antibody Addition:

Dilute 1 μ l conjugated secondary antibody in 10ml BSA blocking buffer and add 100 μ l to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μ l wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation:

Add 100 μ l 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 50 μ l 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

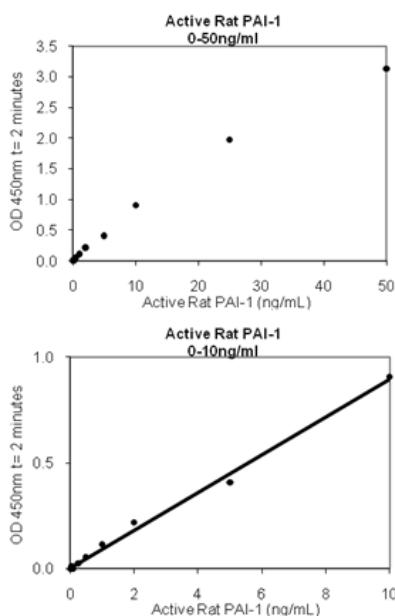
Measurement:

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 (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 concentration level of PAI-1 activity in rat plasma was found to be 1.0 +/- 0.5 ng/ml and 1.8+/-0.9 ng/ml for PAI-1 antigen [8].

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 24 reps of So and 24 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.

References:

1. Thornton AJ, *et al.*: Plasminogen activator inhibitor-1 is an immediate early response gene in regenerating rat liver. *Cancer Research*, **54(5)**: 1337-1343, 1994.
2. Hamdan, Allen D., *et al.*: Angiotensin-Converting Enzyme Inhibition Suppresses Plasminogen Activator Inhibitor-1 Expression in the Neointima of Balloon-Injured Rat Aorta. *Circulation*, **93**: 1073-1078, 1996.
3. DeYoung Mary Beth, *et al.*: Plasminogen Activator Inhibitor Type 1 Increases Neointima Formation in Balloon-Injured Rat Carotid Arteries. *Circulation*, Oct. **104**: 1971-1972, 2001.



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4. Hasenstab David *et al.*: Local Plasminogen Activator Inhibitor Type 1 Overexpression in Rat Carotid Artery Enhances Thrombosis and Endothelial Regeneration While Inhibiting Intimal Thickening. *Arterioscler Thromb Vasc Biol*, Mar **20**: 853-859, 2000.
5. Hagiwara H, *et al.*: Expression of type-1 plasminogen activator inhibitor in the kidney of diabetic rat models. *Thromb Res*, **111**(4-5): 301-9, 2003.
6. Barnes JL, *et al.*: Expression of plasminogen activator-inhibitor-1 (PAI-1) during cellular remodeling in proliferative glomerulonephritis in the rat. *J. Histochem. Cytochem.* Sept.1;**43**(9): 895-905, 1995.
7. van Leeuwen RT, *et al.*: Angiotensin II increases plasminogen activator inhibitor type 1 and tissue-type plasminogen activator messenger RNA in cultured rat aortic smooth muscle cells. *Circulation*, **90**: 362-368, 1994.
8. Thu-Hoa Ngo, *et al.*: Monoclonal antibody-based immunoassays for the specific quantitation of rat PAI-1 antigen and activity in biological samples. *Thromb Haemost*, **79**: 808-12, 1998.

Example of Plate Layout
Samples: 74 wells

Standards: 22 wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.05ng/ml	0.1ng/ml	0.25ng/ml	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml	25ng/ml	50ng/ml	
B	0	0.05ng/ml	0.1ng/ml	0.25ng/ml	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml	25ng/ml	50ng/ml	
C												
D												
E												
F												
G												
H												

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