

Rabbit PAI-1 activity assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

Rabbit PAI-1 activity assay is intended for the quantitative determination of active plasminogen activator inhibitor type-1 in rabbit plasma.

BACKGROUND

PAI-1 plays an important role in fibrinolysis and is a reported risk factor for cardiovascular disease [4]. Increased levels of PAI-1 tend to augment in the presence of proinsulin and insulin [3,5].

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 conjugated secondary antibody. Following an additional washing step, TMB substrate solution 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 plate coated, blocked, and dried with uPA

◆ **Rabbit PAI-1 activity standard:**

1 vial of lyophilized rabbit (wt) PAI-1

◆ **10X Wash Buffer:**

1 bottle of 50ml wash; bring to 1X using DI water

◆ **Anti-human PAI-1 primary antibody:**

1 vial lyophilized monoclonal anti-human PAI-1 antibody

◆ **Primary antibody diluent:**

1 bottle of 10ml diluent

◆ **Horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody

◆ **TMB One substrate solution:**

1 bottle 10 ml 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 100-1000µl.
- 12-channel pipette for 30-300µl
- Paper towels or kimwipes

- 50ml tubes
- 1N H₂SO₄
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- TBS buffer
- 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 (BSA)**

SPECIMEN COLLECTION

Collect 9 volumes of blood in 1 volume of 0.1M trisodium citrate or acidified citrate, preferably using Stabilyte™ evacuated vials (Biopool, cat# 102080). 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. The plasma must be transferred to a clean plastic tube and stored on ice prior to analysis. The PAI-1 activity samples collected in the Stabilyte media 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:

Prepare the PAI-1 standard according to the dilution table:

NOTE: DILUTIONS FOR THE STANDARD CURVE MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

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

Dilution table for preparation of rabbit PAI standard:

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)

Standard and Unknown Addition:

Remove microtiter plate from bag and add 100µl PAI-1 standards (enough for duplicates) 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 3% BSA blocking buffer.

Primary Antibody Addition:

Add 10ml primary antibody diluent directly to the primary antibody 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.

Horseradish peroxidase conjugate

Secondary Antibody Addition:

Dilute 1µl conjugated secondary antibody in 10ml BSA blocking buffer. 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 to all wells and shake plate for 2-10 minutes. Quench the reaction with the addition of 50µl of 1M H₂SO₄ and read final absorbance values at 450nm.

NOTE: Time for substrate development is dependent on needs of researcher.

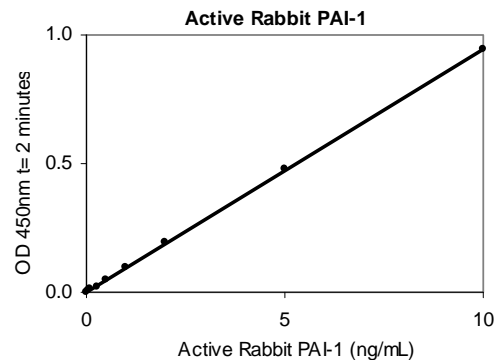
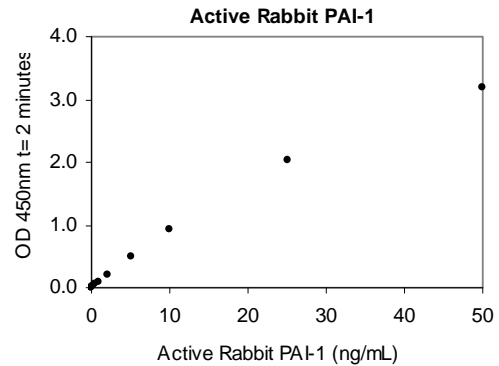
Measurement:

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

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)



EXPECTED VALUES

The concentration level of PAI-1 activity was reported to be 20.5±13.5ng/ml in rabbit plasma and 2.9±2.0ng/ml in rabbit serum [6].

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

- ◆ Endotoxemia: Endotoxin induces a time dependent increase in both PAI-1 antigen and activity levels (40- to 90-fold) [2,6].

◆ Nitrate treatments: Sodium nitroprusside (NP) is reported to inhibit the release of PAI-1 from platelets [1].

◆ Hyperinsulinemia: Increased levels of proinsulin and insulin in plasma increase PAI-1 activity levels [3,5].

vivo. J Mol Cell Cardiol., Aug;**30**(8):1535-43,1998.

6. Ngo TH, *et al*: Immunological quantitation of rabbit plasminogen activator inhibitor-1 in biological samples: evidence that rabbit platelets do not contain PAI-1. Thromb Haemost, Nov;**82**(5):1510-5,1999.

PERFORMANCE CHARACTERISTICS

The assay measures active PAI-1 in the 0.05-50 ng/ml range. Samples giving PAI-1 levels above 50 ng/ml should be diluted in 3% BSA blocking buffer.

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. Lidbury PS, *et al*: Sodium nitroprusside modulates the fibrinolytic system in the rabbit. Br J Pharmacol, Nov;**101**(3):527-30,1990.
2. Krishnamurti C., *et al*: Enhancement of tissue plasminogen activator-induced fibrinolysis by activated protein C in endotoxin-treated rabbits. J. Lab Clin Med., Dec;**118**(6):523-30,1991.
3. Nordt TK, *et al*: Induction of plasminogen activator inhibitor type-1 (PAI-1) by proinsulin and insulin in vivo. Circulation., Feb.1;**91**(3):764-70,1995.
4. Debrock S., *et al*: Neutralization of plasminogen activator inhibitor-1 inhibitory properties: identification of two different mechanisms. Biochim Biophys Acta., Feb 8; **1337**(2):257-66,1997.
5. Nordt TK, *et al*: Augmentation of arterial endothelial cell expression of the plasminogen activator inhibitor type-1 (PAI-1) gene by proinsulin and insulin in