Catalog # PoPAIKT

Porcine PAI-1 activity assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

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

BACKGROUND

PAI-1 is involved in regulating the blood fibrinolytic system. Increased plasma level of PAI-1 is involved in the impairment of fibrinolytic function and may be associated with thrombotic diseases [2]. Increased levels of PAI-1 tend to augment in the presence of insulin [3].

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 strip plate coated with uPA, blocked, and dried
- **10X Wash Buffer:** 1 bottle of 50ml wash; bring to 1X using DI water
- **Porcine PAI-1 activity standard:** 1 vial of lyophilized porcine PAI-1
- **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 of 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 500-5000µl
- Paper towels or kimwipes

- 50ml tubes
- 1N H_2SO_4
- 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

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 [1,4,6]. 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:

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

Dilution table for preparation of pig 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)

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 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.

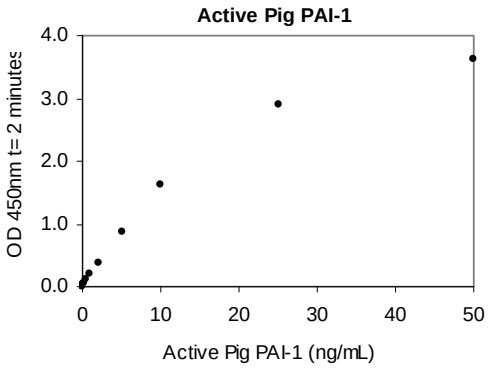
Secondary Antibody Addition:
Dilute 3µl conjugated secondary antibody in 10ml BSA blocking buffer. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

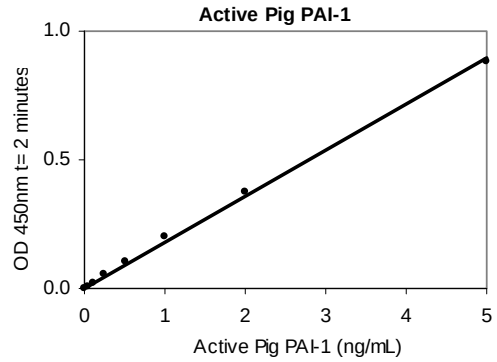
Substrate Incubation:
Add 100µl of substrate solution to all wells and shake plate for 2-10 minutes. Quench the reaction with the addition of 50µl of 1N H_2SO_4 and read final absorbance values at 450nm.
NOTE: Time for substrate development is dependent on needs of researcher.

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 concentration level of PAI-1 activity in male porcine plasma was reported to be 34+/-16 ng/ml and in female porcine plasma 42+/-17 ng/ml [4].

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

♦ Endotoxemia: Endotoxin induces a time dependent increase in PAI-1 activity levels (14-fold increase in 4.5 hours) [7].

♦Thrombotic disease: Increased levels of PAI-1 are reported higher than in normal plasma [2].

♦Hyperinsulinemia: Increased levels of and insulin in plasma increase PAI-1 activity levels [3].

♦Hypercholesterolemia: A cholesterol-rich diet induces an increase in vascular PAI-1 [5].

PERFORMANCE CHARACTERISTICS

The assay measures active PAI-1 in the 0-50 ng/ml range. Samples giving PAI-1 levels above 50ng/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. Lang IM, *et al.*: Presence of active and latent type 1 plasminogen activator inhibitor associated with porcine platelets. Blood, Nov 1;**80**(9):2269-74,1992.

2. Fay WP, *et al.*: High concentrations of active plasminogen activator inhibitor-1 in porcine coronary artery thrombi. Arterioscler Thromb Vasc Biol., Oct;**16**(10):1277-84,1996.

3. Schneider DJ, *et al*: Dependence of augmentation of arterial endothelial cell expression of plasminogen activator inhibitor type 1 by insuliln on soluble factors released from vascular smooth muscle cells. Circulation., Nov4;**96**(9):2868-76,1997.

4. Leng HM., *et al*: Immunoassays for the quantitation of porcine PAI-1 antigen and activity in biological fluid samples. Thromb Haemost, Dec;**84**(6):1082-6,2000.

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6. Fay WP, *et al*: Platelet plasminogen activator inhibitor: purification and characterization of interaction with plasminogen activators and activated protein C. Biochemistry, Jul 11;**28**(14):5773-8,1989.

7. Kutzsche S, *et al*: Hemodynamic changes and systemic activation of coagulation and fibrinolysis during controlled endotoxemia in pigs. Thromb Res., Jun15;**98**(6):513-29,2000.