Porcine PAI-1 Activity ELISA Kit

Catalog # POPAIKT
Strip well format. Reagents for up to 96 tests.
Rev: August 2017

INTENDED USE

This porcine PAI-1 activity assay is intended for the quantitative determination of active plasminogen activator inhibitor type 1 (PAI-1) in porcine plasma, serum and other biological fluids. **For research use only.**

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. After appropriate washing steps, monoclonal anti-porcine PAI-1 primary antibody binds to the PAI-1. Excess antibody is washed away and bound polyclonal antibody is then reacted with the secondary antibody conjugated to horseradish peroxidase. 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

- **96-well urokinase coated microtiter strip plate** (removable wells 8x12) containing urokinase, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Porcine PAI-1 standard:** 1 vial lyophilized standard
- **Anti-PAI-1 primary antibody:** 1 vial lyophilized monoclonal antibody
- **Anti-mouse horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- 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.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS
- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

SAMPLE COLLECTION
Collect 9 volumes of blood in 1 volume of 0.1M trisodium citrate, acidified citrate, or EDTA. 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. Collected samples should be 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 by adding 5ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 50ng/ml standard solution.

Dilution table for preparation of porcine PAI-1 standard:

PAI-1 concentration (ng/ml)	Dilutions
20	600µl (BB) + 400µl (from vial)
10	500µl (BB) + 500µl (20ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2	600µl (BB) + 400µl (5ng/ml)
1	500µl (BB) + 500µl (2ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.2	600µl (BB) + 400µl (0.5ng/ml)
0.1	500µl (BB) + 500µl (0.2ng/ml)
0.05	500µl (BB) + 500µl (0.1ng/ml)
0	500µl (BB) 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 and add 100µl 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: The assay measures PAI-1 antigen in the 0.05-20 ng/ml range. 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. Citrate plasma samples should be applied directly to the plate without dilution. A 1:4 dilution for EDTA plasma and 1:100 dilution for serum is suggested for best results.

Primary Antibody Addition
Reconstitute primary antibody by adding 11ml of blocking buffer directly to the 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
Briefly centrifuge vial before opening. Dilute 2.5µl of conjugated secondary antibody in 10ml of 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 3-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl 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 by gently shaking the plate.

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}).

Calculation of Results

Plot A$_{450}$ against the amount of PAI-1 in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of PAI-1 in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):

Active Pig PAI-1 Full Range

Active Pig PAI-1 Linear Range
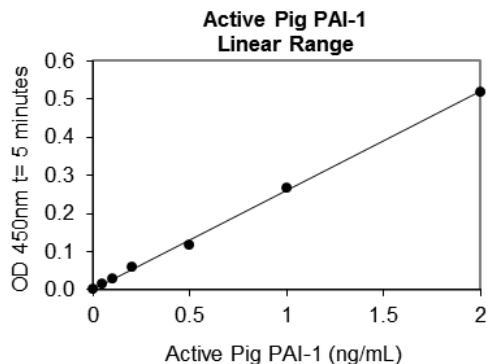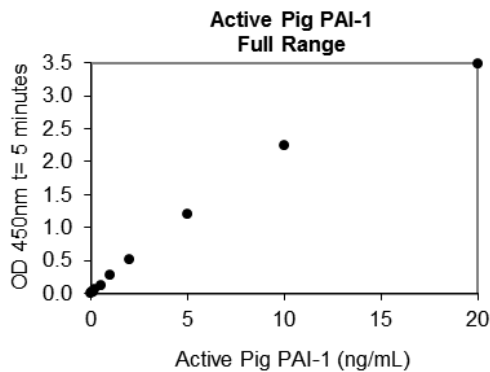
EXPECTED VALUES

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

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD450: 0.067-0.087) and calculating the corresponding concentration. The MDD was 0.046 ng/ml.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Specificity: These studies are currently in progress. Please contact us for more information.

Sample Values: Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean (ng/mL)
Citrate Plasma	Undiluted	0.6
EDTA Plasma	1:4	16.5
Serum	1:100	67.3

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 of or contact with the above product.

REFERENCES

1. Lang IM, *et al.*: Blood. 1992, 80(9):2269-74.
2. Fay WP, *et al.*: Arterioscler Thromb Vasc Biol. 1996, 16(10):1277-84.
3. Schneider DJ, *et al.*: Circulation. 1997, 96(9):2868-76.
4. Leng HM., *et al.*: Thromb Haemost. 2000, 84(6):1082-6.
5. Orbe J, *et al.*: Cardiovasc Res. 2001, 49(2):484-92.
6. Fay WP, *et al.*: Biochemistry. 1989, 28(14):5773-8.
7. Kutzsche S, *et al.*: Thromb Res. 2000, 98(6):513-29.

Example of ELISA Plate Layout
96 Well Plate: 20 Standard wells, 76 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml		
B	0	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml		
C												
D												
E												
F												
G												
H												