LSBio™ Porcine SERPINE1 / PAI-1 Enzyme Capture ELISA Kit

Catalog No. LS-F10472

User Manual

Please Read the Manual Carefully
Before Starting your Experiment

LSBio
LifeSpan BioSciences, Inc.

For research use only. Not approved for use in humans or for clinical diagnosis.

LSBio
LifeSpan BioSciences, Inc.
www.lsbio.com

Porcine PAI-1 Activity ELISA Kit

Catalog # LS-F10472 Strip well format. Reagents for up to 96 tests.

Rev:December 2014

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 3.5ml 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 (25ng/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. A 1:10 dilution is recommended for normal porcine plasma.

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.

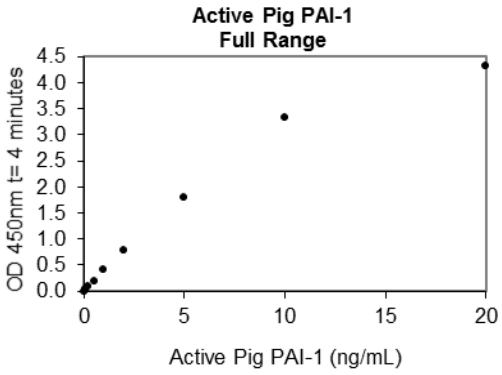
Measurement

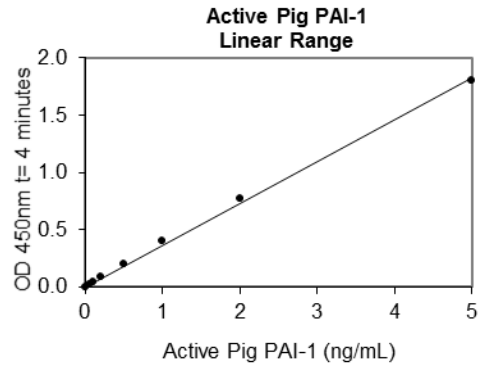
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

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):





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

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: To assess the linearity of the assay, pooled citrated porcine plasma samples were serially diluted in blocking buffer to produce samples with values within the dynamic range of the assay.

Plasma Dilution	Dilution Corrected Value (ng/mL)	% Change From Previous Dilution
Neat	16.7975	--
1:2	25.8242	153.7384
1:4	33.6312	130.2313
1:8	34.4504	102.4358
1:16	35.4144	102.7982
1:32	35.4304	100.0452

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)
EDTA Plasma	1:4	16.5
	1:8	17.9
	1:16	18.2
	1:32	19.3

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												

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion.

Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

Returns, Refunds, Cancelations: Any problems with LifeSpan products must be reported to LifeSpan within 10 days of product receipt. The customer must obtain written authorization from LifeSpan before returning items. To request that goods be returned, please contact LifeSpan Technical Support. If an error by LifeSpan BioSciences results in shipment of an incorrect order, LifeSpan will, at its option, either ship a replacement order at no charge, or credit the customer's account for the original product shipped in error. Returns and cancelations may be subject to a 30% restocking fee.

Conditions & Warranty: All LifeSpan products are intended for Research Use Only and are not for use in human therapeutic or diagnostic applications. The information supplied with each product is believed to be accurate, but no warranty or guarantee is offered for the products, because the ultimate conditions of use are beyond LifeSpan's control. The information supplied with each product is not to be construed as a recommendation to use this product in violation of any patent, and LifeSpan will not be held responsible for any infringement or other violation that may occur with the use of its products. Under no event will LifeSpan be responsible for any loss of profit or indirect consequential damage, including, but not limited to, personal injuries resulting from use of these products. LifeSpan's liability to any user of Products for damages that do not result from any fault of the user, will be limited to replacement of the Product(s) only, and in no event shall LifeSpan's liability exceed the actual price received by LifeSpan for the Product(s) at issue. LifeSpan shall not be liable for any indirect, special, incidental or consequential damages. LIFESPAN FURTHER DISCLAIMS ANY AND ALL EXPRESS AND IMPLIED OR STATUTORY WARRANTIES WITH RESPECT TO THE PRODUCTS, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE. LifeSpan disclaims any and all responsibility for any injury or damage which may be caused by the fault of the user.

For research use only. Not approved for use in humans or for clinical diagnosis.

LSBio
LifeSpan BioSciences, Inc.

2401 Fourth Avenue Suite 900 Seattle, WA 98121
Tel: 206.374.1102
Fax: 206.577.4565

Technical.Support@LSBio.com