

# **LSBio™ Rat SERPINE1 / PAI-1 ELISA Kit**

**Catalog No. LS-F10460**

## **User Manual**

**Please Read the Manual Carefully  
Before Starting your Experiment**



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

## INTENDED USE

This rat plasminogen activator inhibitor type 1 (PAI-1) total antigen assay is intended for the quantitative determination of total PAI-1 in biological fluids.

**For research use only.**

## BACKGROUND

Plasminogen activator inhibitor-1 (PAI-1) is a central regulator of the blood fibrinolytic system [1]. Clinical studies have indicated that increased PAI-1 levels increase the risk for thrombosis, whereas decreased levels may cause recurrent bleeding [2].

## ASSAY PRINCIPLE

Rat PAI-1 present in samples reacts with the capture antibody coated on the microtiter plate. Free, latent, and complexed PAI-1 will bind to the plate. After appropriate washing steps, primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with horseradish peroxidase conjugated secondary antibody. TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of rat PAI-1. The amount of color development is directly proportional to the concentration of total PAI-1 in the sample.

## REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-rat PAI-1 antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Rat PAI-1 standard:** 1 vial lyophilized standard
- **Anti-rat PAI-1 primary antibody:** 1 vial lyophilized polyclonal antibody
- **Anti-rabbit 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 standards 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<sub>2</sub>SO<sub>4</sub> 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 plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles. Samples of rat serum, urine, cell culture media, or tissue extracts may also be used.

## 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 rat PAI-1 standard:

PAI-1 concentration (ng/ml)	Dilutions
50	100 $\mu\text{l}$ from standard vial
25	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (50ng/ml)
10	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (25ng/ml)
5	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (10ng/ml)
2	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (5ng/ml)
1	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (2ng/ml)
0.5	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (1ng/ml)
0.25	500 $\mu\text{l}$ (BB) + 500 $\mu\text{l}$ (0.5ng/ml)
0.1	600 $\mu\text{l}$ (BB) + 400 $\mu\text{l}$ (0.25ng/ml)
0	500 $\mu\text{l}$ (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 100 $\mu\text{l}$  of 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 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures total PAI-1 in the 0.1-50 ng/ml range. Plasma and serum samples should be applied directly to the plate without dilution. If the unknown is thought to have high PAI-1 levels, dilutions may be made in plasma devoid of PAI-1 (Cat# RPLA-SC-PAI) or in blocking buffer.

### Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100 $\mu\text{l}$  to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

### Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 1 $\mu\text{l}$  of HRP conjugated secondary antibody in 10ml of blocking buffer and add 100 $\mu\text{l}$  to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 $\mu\text{l}$  wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

### Substrate Incubation

Add 100 $\mu\text{l}$  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 $\mu\text{l}$  of 1N  $\text{H}_2\text{SO}_4$  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.

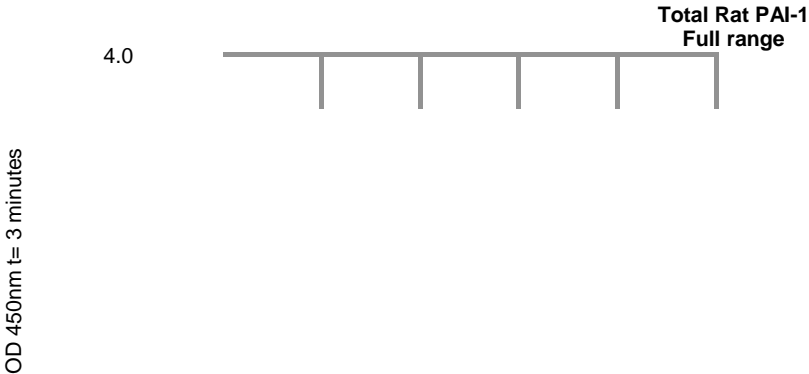
### Measurement

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):



**Linearity:** To assess the linearity of the assay, rat plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	99	102	101	100
Range	98-102%	95-108%	99-102%	93-105%

**Specificity:** This assay recognizes natural and recombinant total rat PAI-1. Pooled normal plasma from pig, horse and dog was assayed and significant cross-reactivity was observed. Pooled normal plasma from mouse, human and cyno monkey was assayed and minor cross-reactivity was observed. Pooled normal plasma from sheep was assayed and no significant cross-reactivity was observed. Pooled normal plasma from rabbit resulted in significant color development.

**Sample Values:** Samples were evaluated for the presence of the antigen.

Sample Type	Dilution	Mean (ng/mL)
Citrate Plasma	Undiluted	2.0

**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	0.1 ng/ml	0.25 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml		
B	0	0.1 ng/ml	0.25 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	25 ng/ml	50 ng/ml		
C												
D												
E												
F												
G												
H												

**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. Eitzman DT, *et al.*: Blood. 2000, 95(2): 577-580.
2. Kawasaki T, *et al.*: Blood. 2000, 96(1): 153-160.
3. Ngo TH, *et al.*: Thromb Haemostas. 1998, 79(4): 808-812.
4. Schafer K, *et al.*: FASEB J. 2001, 15: 1840-1842.
5. Samad F, *et al.*: PNAS. 1996, 96(12): 6902-6907.
6. Seki T, *et al.*: Thromb Haemostas. 1996, 75(5): 801-807.





**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.

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