Mouse PAI-1 ELISA Kit

Catalog No.: IMPAIKT   **Lot No. 413**

Mouse PAI-1 activity assay is intended for the quantitative determination of active plasminogen activator inhibitor type 1 in mouse plasma. For research use only.

Innovative Research, Inc

INTENDED USE
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BACKGROUND
Plasminogen activator inhibitor-1 (PAI-1) is a central regulator of the blood fibrinolytic system [2]. Clinical studies have indicated that increased PAI-1 levels increase the risk for thrombosis, whereas decreased levels may cause recurrent bleeding [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 secondary antibody. Following an additional washing step, TMB 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 uPA coated microtiter strip plate** (removable wells 8x12) containing uPA, blocked and dried.
·**10X Wash Buffer:** 1 bottle of 50ml
·**Mouse PAI-1 activity standard:** 1 vial lyophilized standard
·**Anti-mouse 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_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 a 3.8% trisodium citrate or acidified citrate. 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 [4]. The plasma must be stored on ice prior to analysis. The PAI-1 activity samples collected 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 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 mouse PAI-1 standard:
NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

PAI-1 concentration (ng/ml)	Dilutions
50	100µl from standard vial
25	500µl (BB) + 500µl (50ng/ml)
10	600µl (BB) + 400µl (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)
0.25	500µl (BB) + 500µl (0.5ng/ml)
0.1	600µl (BB) + 400µl (0.25ng/ml)
0.05	500µl (BB) + 500µl (0.1ng/ml)
0	500µl (BB) Zero point to determine background

Standard and Unknown Addition

Remove microtiter plate from bag and add 100µl PAI-1 activity 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 activity levels in the 0.05-50 ng/ml range. If the unknown is thought to have high PAI-1 activity levels, dilutions may be made 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 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 1µl 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 2-10 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. For best results read plate immediately. 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 activity 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):
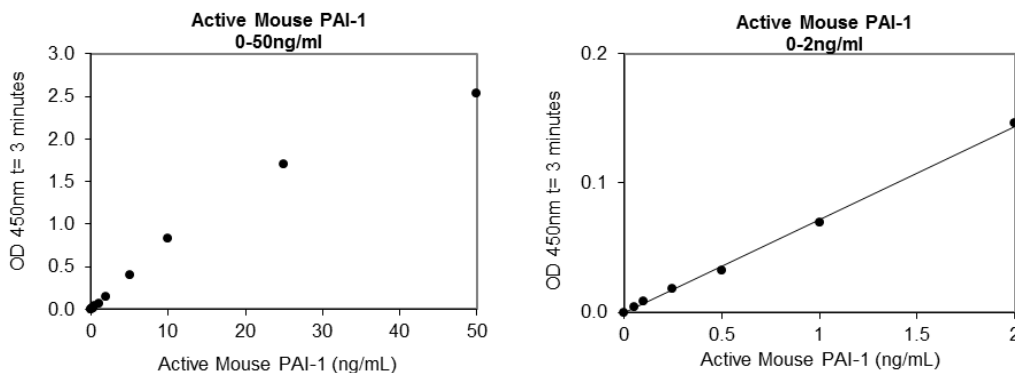
EXPECTED VALUES

The concentration level of PAI-1 in mouse plasma was found to be 1.9±0.6 ng/ml [1]. Abnormalities in PAI-1 levels have been reported in the following conditions:

·Endotoxemia: Endotoxin induces a large increase in PAI-1 levels (80-fold) [1].
·Hyperglycemia, hyperinsulinemia, and insulin resistance: Elevated PAI-1 levels in obese and diabetic mice contribute to these metabolic disorders [5,6].
·Vascular thrombosis: Increased PAI-1 levels may contribute to venous thrombosis [2].
·Myocardial Infarction: Increased PAI-1 levels may contribute to myocardial infarction [2].

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: 0.086-0.097) and calculating the corresponding concentration. The MDD was 0.031 ng/ml.

Intra-assay Precision: Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	1.80	3.15	4.58
Standard Deviation	0.116	0.210	0.368
CV (%)	6.47	6.67	8.03

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

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in blocking buffer was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/ml)	0.468	1.79	3.96	12.6
Average % Recovery	109	113	100	92
Range	105-113%	108-118%	99-101%	88-96%

Linearity: To assess the linearity of the assay, mouse 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	95	102	101	117
Range	91-98%	96-104%	94-106%	114-118%

Specificity: This assay recognizes natural active mouse PAI-1. Pooled normal plasma from human, rat, pig, sheep, canine, and guinea pig were assayed and no significant cross-reactivity was observed. Pooled normal plasma from rabbit resulted in significant color development. The following factors were prepared in blocking buffer and assayed for cross-reactivity.

Recombinant Rat PAI-1	Cross-reacts 29%
Recombinant Rabbit PAI-1	Cross-reacts 18%
Recombinant Porcine PAI-1	Cross-reacts 1%
Recombinant Human PAI-1	Cross-reacts 76%

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

Sample Type	Active PAI-1	Total PAI-1 (ng/ml)
Citrate Plasma	1.61	23.7
CD-1 Plasma	1.03	17.6

Example of ELISA Plate Layout
96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.05 ng/ml	0.1 ng/ml	0.25 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	0.05 ng/ml	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												