

## Murine PAI-1 Total Antigen Assay

Strip well format. Reagents for up to 96 tests.

### For Research Use Only.

#### INTENDED USE

Murine PAI-1 total assay is intended for the quantitative determination of total plasminogen activator inhibitor type 1 in mouse plasma.

#### 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

Murine PAI-1 present in plasma reacts with the capture antibody coated and dried on a microtiter plate. Free, latent, and complexed PAI-1 will bind to the plate. Any unbound PAI-1 is 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 total PAI-1 present in the samples, is then reacted with the 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 total PAI-1 in the sample.

#### REAGENTS PROVIDED

◆ **96-well antibody coated microtiter strip plate:** containing mouse anti-mouse PAI-1 antibody dried and blocked on the strip well surface.

◆ **10X Wash Buffer:**

1 bottle of 50ml; bring to 1X using DI water

◆ **Murine PAI-1 activity standard:**

1 vial lyophilized standard

◆ **Anti-murine PAI-1 primary antibody:**

1 vial lyophilized polyclonal anti-mouse antibody

◆ **Anti-rabbit horseradish peroxidase secondary antibody:**

1 vial concentrated HRP labeled antibody

◆ **TMB substrate solution:**

1 bottle 10ml 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 200-1000µl
- 12-channel pipette covering 50-500µl
- Paper towels or kimwipes
- 50ml tubes
- 1N H<sub>2</sub>SO<sub>4</sub>
- DI water
- Magnetic stirrer and stir-bars

- Plastic containers with lids
- TBS buffer
- 3% 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 [4]. 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.

The assay measures PAI-1 antigen in the 0.05-50 ng/ml range. Samples giving PAI-1 levels above 50ng/ml should be diluted in plasma devoid of active PAI-1.

#### 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 mouse 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 plasma devoid of PAI-1, or in blocking buffer.

**Primary Antibody Addition:**

Reconstitute primary antibody as directed on 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 conjugated secondary antibody in 10ml of 3% BSA 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 of substrate solution to all wells and shake plate at 300rpm for 2-10 minutes. Quench the reaction with the addition of 50µl of 1N H<sub>2</sub>SO<sub>4</sub> and read final absorbance values at 450nm. NOTE: Time for substrate development is dependent on needs of researcher.

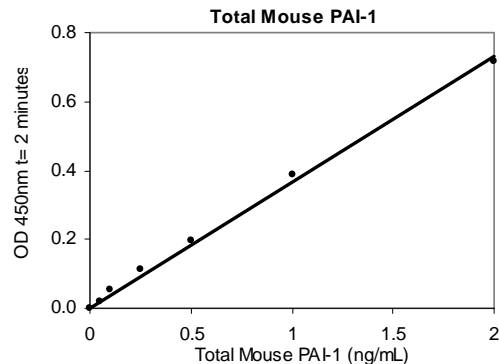
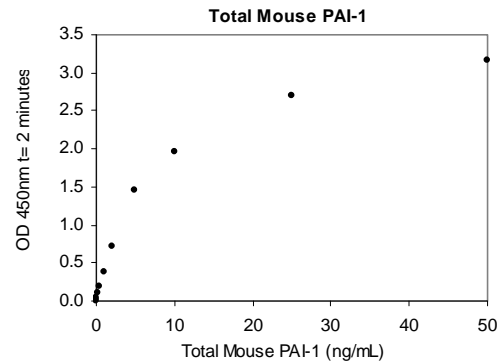
**Measurement:**

Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm, A<sub>450</sub>.

**Assay Calibration:**

Plot A<sub>450</sub> 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 antigen in murine plasma was found to be 1.9 +/- 0.6 ng/ml [1]. Abnormalities in PAI-1 levels have been reported in the following condition:

- ◆ 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 [7].
- ◆ Myocardial Infarction: Increased PAI-1 levels may contribute to myocardial infarction [7].

#### PERFORMANCE CHARACTERISTICS

##### **Sensitivity = 0.032 ng/ml**

(calculated by determining the OD of 20 reps of So and 20 reps of the low standard)

##### **Linearity**

The slope = 0.9315

Correlation coefficient = 0.9998

##### **Intra Assay Precision**

High 2.6%, Medium 5.2%, Low 7.2%

(calculated by running 20 reps of each concentration in an assay)

biologic fluids with a murine monoclonal antibody-based enzyme-linked immunosorbent assay. *Blood*, **71(1)**: 220-225, 1988.

5. Schafer, Katrin, *et al.*: Disruption of the plasminogen activator inhibitor 1 gene reduces the adiposity and improves the metabolic profile of genetically obese and diabetic ob/ob mice. *FASEB*, Aug**15**: 1840-2, 2001.

6. Samad F., *et al.*: Tumor necrosis factor alpha is a key component in the obesity-linked elevation of plasminogen activator inhibitor 1. *Proc Natl Acad Sci USA*, Jun8;**96(12)**: 6902-7, 1996.

7. Eitzman, Daniel T., *et al.*: Plasminogen activator inhibitor-1 and vitronectin promote vascular thrombosis in mice. *Blood*, Jan15;**95(2)**: 577-580, 2000.

#### 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.

#### REFERENCE

1. Declerck PJ, *et al.*: Immunoassay of murine t-PA, u-PA, and PAI-1 using monoclonal antibodies raised in gene-inactivated mice. *Thromb Haemostas.*, Nov;**74(5)**: 1305-9, 1995.
2. Eitzman Daniel T., *et al.*: Plasminogen activator inhibitor-1 and vitronectin promote vascular thrombosis in mice. *Blood*, Jan;**95(2)**: 577-580, 2000.
3. Kawasaki Tomihisa, *et al.*: Vascular release of plasminogen activator inhibitor-1 impairs fibrinolysis during acute arterial thrombosis in mice. *Blood*, July;**96(1)**: 153-160, 2000.
4. Declerck PJ, *et al.*: Measurement of plasminogen activator inhibitor 1 in