

Enzyme Immunoassay for

Rat Plasminogen Activator Inhibitor (PAI-1) Total Antigen

For Research Use Only

INTRODUCTION

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.

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).

PRINCIPLES OF PROCEDURE

Rat PAI-1 will bind to the capture antibody coated on the microtiter plate. Free, latent, and complexed PAI-1 will react with the capture antibody on 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 a secondary antibody. Following an additional washing step, TMB is then used for color development at 450 nm. The amount of color development is directly proportional to the concentration of total PAI-1 in the sample.

MATERIALS PROVIDED

Component	Contents	Quantity	Storage	Cat. No.
Coated Plate	Anti-rat PAI-1 coated 96-well plate	1 plate	4°C	PI98a
Standard	Rat PAI-1 activity standard	1 vial	4°C	PI98b
Primary Antibody	Rabbit anti-rat PAI-1 antibody	1 vial	4°C	PI98c
Wash Buffer	10x solution for washing plate	50 mL	4°C	PI98d
Secondary Antibody	Anti-rabbit HRP conjugated antibody	1 vial	4°C	PI98e
Substrate	TMB Substrate	10 mL	4°C	PI98f

MATERIALS NEEDED BUT NOT PROVIDED

1. Pipettes covering 0-10 μ l and 200-1000 μ l and tips
2. 12-channel pipette covering 30-300 μ l
3. 1N H₂SO₄
4. DI water
5. Microtiter plate spectrophotometer with a 450 nm filter
6. Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm

STORAGE CONDITIONS

1. Store this kit and its components at 4°C until use.
2. 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.

PROCEDURAL NOTES

1. Use aseptic technique when opening and dispensing reagents.
2. This kit is designed to work properly as provided and instructed. Additions, deletions, or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.
3. Exercise universal precautions during the performance or handling of this kit or any component contained therein.

SAMPLE COLLECTION AND PREPARATION

1. Samples of rat plasma, serum, urine, cell culture media, or tissue extracts may be applied directly to the plate.
2. The assay measures total PAI-1 in the 0.05-50 ng/ml range. Samples giving PAI-1 levels above 50 ng/ml should be diluted in a similar biological fluid devoid of PAI-1 or 3% BSA Blocking Buffer.

REAGENT PREPARATION

1. Dilute the 50 mL of 10x Wash Buffer concentrate to 1x with 450 mL of DI water prior to use.
2. Prepare 100 mL of TBS Buffer: 0.1 M Tris-HCL, 0.15 M NaCl, pH 7.4
3. Prepare 20 mL of 3% BSA Blocking Buffer: 3% BSA in TBS Buffer.

STANDARD PREPARATION

Reconstitute the Standard as directed on the vial to give a 50 ng/mL Standard Stock Solution. **Do not prepare the Standards until you are ready to apply them to the plate.**

Table 1: Preparation of Standard Curve

Standard	PAI-1 Concentration (ng/mL)	Blocking Buffer (μ L)	Transfer Volume (μ L)	Transfer Source	Final Volume (μ L)
S ₁₀	50	0	1000	Stock Vial	500
S ₉	25	600	400	S ₁₀	600
S ₈	10	500	500	S ₉	500
S ₇	5	500	500	S ₈	600
S ₆	2.5	500	500	S ₇	500
S ₅	1	600	400	S ₆	500
S ₄	0.5	500	500	S ₅	500
S ₃	0.2	600	400	S ₄	600
S ₂	0.1	500	500	S ₃	500
S ₁	0.05	500	500	S ₂	1000

ASSAY PROCEDURE

1. Add 100 μ l of the Standards and unknowns to the wells in duplicate. If the unknown is thought to have high PAI-1 levels, dilutions may be made in plasma devoid of PAI-1, or in 3% BSA Blocking Buffer. For a suggested plate layout, see Scheme I on the following page.
2. Shake the plate at 300 rpm for 30 minutes at room temperature.
3. Wash the plate three times with 300 μ L of Wash Buffer. Remove excess Wash Buffer by gently tapping the plate on a paper towel.
4. Reconstitute the Primary Antibody as directed on the vial and agitate gently to completely dissolve contents. Add 100 μ l to each well.

5. Shake the plate at 300 rpm for 30 minutes at room temperature.
6. Wash the plate three times with 300 μL of Wash Buffer. Remove excess Wash Buffer by gently tapping the plate on a paper towel.
7. Dilute the Secondary Antibody in 3% BSA Blocking Buffer as directed on the vial and add 100 μl to each well.
8. Shake the plate at 300 rpm for 30 minutes at room temperature.
9. Wash the plate three times with 300 μL of Wash Buffer. Remove excess Wash Buffer by gently tapping the plate on a paper towel.
10. Add 100 μl of TMB Substrate to each well.
11. Shake the plate at 300 rpm for 2-10 minutes.
12. Stop the reaction with 50 μl of 1N H_2SO_4 and read the plate at 450 nm.

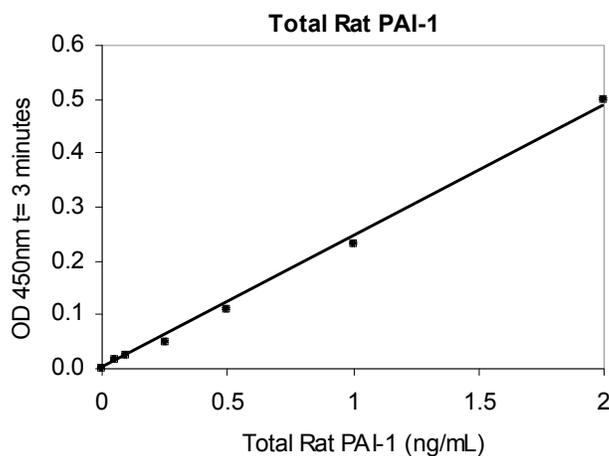
Scheme I:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S10	S10	S2	S2	U7	U7	U15	U15	U23	U23	U31	U31
B	S9	S9	S1	S1	U8	U8	U16	U16	U24	U24	U32	U32
C	S8	S8	U1	U1	U9	U9	U17	U17	U25	U25	U33	U33
D	S7	S7	U2	U2	U10	U10	U18	U18	U26	U26	U34	U34
E	S6	S6	U3	U3	U11	U11	U19	U19	U27	U27	U35	U35
F	S5	S5	U4	U4	U12	U12	U20	U20	U28	U28	U36	U36
G	S4	S4	U5	U5	U13	U13	U21	U21	U29	U29	U37	U37
H	S3	S3	U6	U6	U14	U14	U22	U22	U30	U30	U38	U38

CALCULATIONS

1. Plot the A_{450} against the concentrations of PAI-1 in the standards.
2. Fit a straight line through the points using a linear fit procedure.
3. Calculate the PAI-1 concentrations of the unknowns using the standard curve.

**Figure 1: Typical Standard Curve
(Do Not Use For Calculations)**



EXPECTED VALUES

The level of PAI-1 antigen in rat plasma was 1.8 +/- 0.9 ng/ml (mean +/- SD, n = 18), with a corresponding value of 1.0 +/- 0.5 ng/ml for PAI-1 activity (3).

Abnormalities in PAI-1 levels have been reported in the following conditions:

- Endotoxemia: Endotoxin induces a large increase in PAI-1 levels (100-200 fold) (3).
- Hyperglycemia, hyperinsulinemia, and insulin resistance: Elevated PAI-1 levels in obese and diabetic mice contribute to these metabolic disorders (4,5).
- Vascular thrombosis: Increased PAI-1 levels may contribute to venous thrombosis (6).
- Myocardial Infarction: Increased PAI-1 levels may contribute to myocardial infarction (6).
- Cirrhosis: Cirrhotic rat liver expressed an increased level of PAI-1 compared to normal liver (7).

REFERENCES

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