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 μl and 200-1000 μl and tips
2. 12-channel pipette covering 30-300μl
3. 1N H_2SO_4
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

Samples of rat plasma, serum, urine, cell culture media, or tissue extracts may be applied directly to the plate.

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 (μL)	Transfer Volume (μL)	Transfer Source	Final Volume (μL)
S_{10}	50	0	100	Stock Vial	500
S_9	25	500	500	S_{10}	600
S_8	10	600	400	S_9	500
S_7	5	500	500	S_8	600
S_6	2	600	400	S_7	500
S_5	1	500	500	S_6	500
S_4	0.5	500	500	S_5	500
S_3	0.25	500	400	S_4	600
S_2	0.1	600	400	S_3	500
S_1	0.05	500	500	S_2	1000

ASSAY PROCEDURE

1. Add 100 μl 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 μL 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 µl 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.

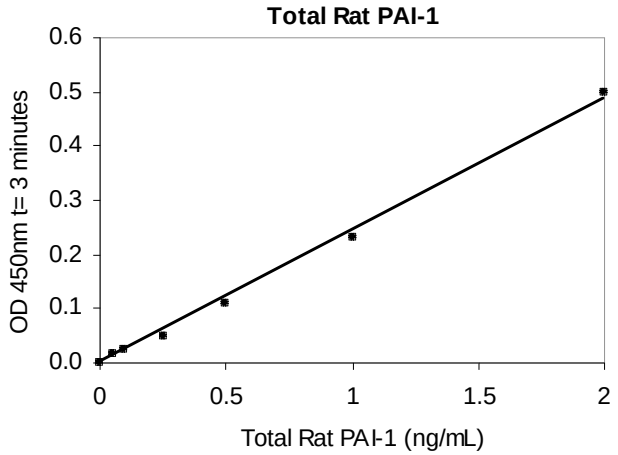
Scheme I:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_{10}	S_{10}	S_2	S_2	U_7	U_7	U_{15}	U_{15}	U_{23}	U_{23}	U_{31}	U_{31}
B	S_9	S_9	S_1	S_1	U_8	U_8	U_{16}	U_{16}	U_{24}	U_{24}	U_{32}	U_{32}
C	S_8	S_8	U_1	U_1	U_9	U_9	U_{17}	U_{17}	U_{25}	U_{25}	U_{33}	U_{33}
D	S_7	S_7	U_2	U_2	U_{10}	U_{10}	U_{18}	U_{18}	U_{26}	U_{26}	U_{34}	U_{34}
E	S_6	S_6	U_3	U_3	U_{11}	U_{11}	U_{19}	U_{19}	U_{27}	U_{27}	U_{35}	U_{35}
F	S_5	S_5	U_4	U_4	U_{12}	U_{12}	U_{20}	U_{20}	U_{28}	U_{28}	U_{36}	U_{36}
G	S_4	S_4	U_5	U_5	U_{13}	U_{13}	U_{21}	U_{21}	U_{29}	U_{29}	U_{37}	U_{37}
H	S_3	S_3	U_6	U_6	U_{14}	U_{14}	U_{22}	U_{22}	U_{30}	U_{30}	U_{38}	U_{38}

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

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

DISCLAIMER

This information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. Oxford Biomedical Research, Inc. shall not be held liable for any damage resulting from handling or from contact with the above product. See catalog for additional terms and conditions of sale.

TECHNICAL SUPPORT

If you need technical information or assistance with assay procedures, please call our Technical Support Department at 800-692-4633 or 248-852-8815. Our staff will answer your questions about this or any other product in the Oxford Biomedical line.

GUARANTEE AND LIMITATION OF REMEDY

Oxford Biomedical Research, Inc. makes no guarantee of any kind, expressed or implied, which extends beyond the description of the material in this ELISA kit, except that these materials and this kit will meet our specifications at the time of delivery. Buyer's remedy and Oxford Biomedical Research, Inc.'s sole liability hereunder is limited to, at Oxford Biomedical Research, Inc.'s option, refund of the purchase price of, or the replacement of, material that does not meet our specification. By acceptance of our products, Buyer indemnifies and holds Oxford Biomedical Research, Inc. harmless against, assumes all liability for the consequence of its use or misuse by the Buyer, its employees, or others. Said refund or replacement is conditioned of Buyer notifying Oxford Biomedical Research, Inc. within thirty (30) days of the receipt of product. Failure of Buyer to give said notice within said thirty (30) days shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s).

Oxford Biomedical Research, Inc.
P.O. Box 522
Oxford, MI 48371 U.S.A.

Orders: 800-692-4633
Technical Service: 248-852-8815
Fax: 248-852-4466
E-mail: info@oxfordbiomed.com

Made in the U.S.A.