Activity Assay for
Rat PAI-1
For Research Use Only

INTRODUCTION

Plasminogen Activator Inhibitor-1 (PAI-1) is a glycoprotein and member of the serine proteinase inhibitor (serpin) superfamily. PAI-1 is the primary inhibitor of tissue-type plasminogen activator (tPA) and the urokinase-type plasminogen activator (uPA). This inhibition exhibits antiproteolytic properties that can lead to myocardial infarction and thromboembolic disease with elevated levels of PAI-1. Additionally, PAI-1 is thought to play a role in the function of tissue remodeling and tumor metastasis.

PRINCIPLES OF PROCEDURE

This is an ELISA (Enzyme-Linked Immunosorbent Assay) for the quantitative analysis of PAI-1 levels in biological fluid. This test kit operates on the basis of sandwich ELISA where active PAI-1 complexes with uPA and is quantified with the use of an HRP labeled secondary antibody.

The functional or active PAI-1 binds to the uPA coated on the well of the microtiter plate. The latent and complexed forms of PAI-1 will not bind and are discarded at a later washing step. Next, PAI-1 primary antibody is added to the wells binding to the captured PAI-1 on the microtiter plate. HRP conjugated secondary antibody is then added for detection of the active PAI-1. Quantitative test results are obtained by the measure and comparison of the sample and standard absorbance readings.

MATERIALS PROVIDED

Component	Contents	Quantity	Storage	Cat. No.
Coated Plate	96-well microplate coated with Anti-PAI-1	1 plate	4°C	PI92a
Standard	Rat PAI-1 activity standard (50 ng/mL)	1 vial	4°C	PI92b
Wash Buffer	10x solution for washing plate	50 mL	4°C	PI92c
Substrate	TMB Substrate	10 mL	4°C	PI92d
Primary Antibody	Anti-rat PAI-1 polyclonal antibody (lyophilized)	1 vial	4°C	PI92e
Secondary Antibody	Anti-rabbit HRP conjugated antibody	1 vial	4°C	PI92f

MATERIALS NEEDED BUT NOT PROVIDED

1. 1 N H_2SO_4
2. TBS Buffer (see Reagent Preparation)
3. Blocking Buffer (see Reagent Preparation)
4. DI Water
5. Microplate reader with 450 nm filter
6. Microplate shaker with uniform horizontal circular movement up to 300 rpm
7. Precision pipettes that range from 10 µL-1000 µL and disposable tips

STORAGE

1. Store the kit and all of its components at 4°C before use.

2. If not using the entire plate at once, prepare only the appropriate amount of Primary Antibody and PAI-1 Standard. The remaining stock solutions should be frozen and stored at –70°C. Primary Antibody should be used within two weeks. All other components should remain refrigerated.
3. Store unused portions of the microplate in a pouch with a desiccant at 4°C.

PROCEDURAL NOTES

1. This assay should be run at room temperature.
2. Use aseptic technique when opening and dispensing reagents.
3. 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.
4. To minimize error due to handling, wipe the exterior bottom of the microplate wells with a lint-free paper towel.

SAMPLE COLLECTION, STORAGE, AND PREPARATION

Blood should be collected in a 9:1 ratio with trisodium citrate or acidified citrate. Immediately after collection, centrifuge the samples at 3000 x *g* for 15 minutes. The plasma must be platelet free as they can release PAI-1. Store the plasma samples on ice prior to analysis. The plasma is stable at -20°C for one month with 3 freeze-thaw cycles.

REAGENT PREPARATION

1. **TBS Buffer:** 0.10 M TRIS, 0.15 M NaCl, pH 7.4
2. **Blocking Buffer:** 3% BSA in TBS Buffer.
3. **10x Wash Buffer:** Dilute the 50 mL of concentrate to 1x with 450 mL of DI water prior to use.
4. **Standard:** Reconstitute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare according to include Standard Dilution Table immediately prior to use.
5. **Primary Antibody:** Reconstitute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use.
6. **Secondary Antibody:** Dilute with 3% BSA Blocking Buffer as directed on the vial and vortex gently to mix. Prepare immediately prior to use.

ASSAY PROCEDURE

1. Add 100 µL of standards or unknowns to each well. See **Scheme I** for a suggested plate layout. Shake the plate at 300 rpm on a plate shaker for 30 minutes.
2. Wash wells according to the following wash procedure:
   a. Remove contents of the plate by inversion into an appropriate disposal device.
   b. Tap out remaining contents of the plate onto a lint free paper towel.
   c. Add 300 µL of Wash Buffer to each well.
   d. Let stand for 2-3 minutes.
   e. Remove contents of the plate by inversion into an appropriate disposal device.
   f. Repeat procedure 2 more times and proceed to step “g”.
   g. Tap out the remaining contents of the plate onto a lint free paper towel and proceed to step 6.
3. Add 100 µL of diluted Primary Antibody to each well. Shake plate at 300 rpm for 30 minutes.
4. Wash wells according to step 2.
5. Add 100 µL of the diluted Secondary Antibody to each well. Shake plate at 300 rpm for 30 minutes.
6. Wash wells according to step 2.
7. Add 100 µL of TMB Substrate to each well and incubate for 2-10 minutes with shaking.
8. Stop the reaction with 50 µL per well of 1 N H_2SO_4 and read plate at 450 nm.

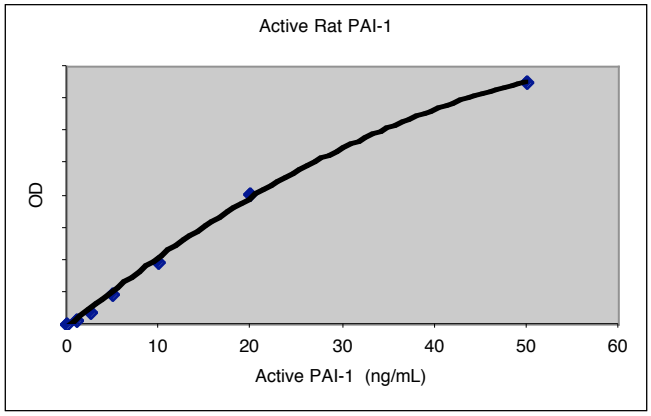
Scheme I:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S_0	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	U_1
B	S_0	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	U_1
C	U_2	U_3	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}
D	U_2	U_3	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}
E	U_{14}	U_{15}	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}
F	U_{14}	U_{15}	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}
G	U_{26}	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}
H	U_{26}	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}

CALCULATIONS

1. Average the O.D. values for each pair of duplicate wells.
2. Plot a standard curve using the average O.D. versus the standard concentration.
3. Fit a straight line through the points using a linear fit procedure.
4. Determine the concentration of each unknown using the equation from the standard curve.

Typical Standard Curve:



PERFORMANCE CHARACTERISTICS

Sensitivity: 0.011 ng/mL

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