Activity Assay for
Human 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 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 will bind 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, a 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	Volume	Storage
uPA Coated Plate		4°C
Human PAI-1 Standard (0 U/mL)	1 vial	4°C
Human PAI-1 Standard (230 U/mL)	1 vial	4°C
10x Wash Buffer	50 mL	4°C
Anti-human PAI-1 Primary Antibody	1 vial	4°C
TMB Substrate	10 mL	4°C
EIA Buffer	10 mL	4°C
HRP Conjugate Secondary Antibody	1 vial	4°C

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 the pouch with a desiccant at 4°C.

WARNINGS AND PRECAUTIONS

1. 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.
2. Exercise universal precautions during the performance or handling of this kit or any component contained therein.

PROCEDURAL NOTES

1. This assay should be run at room temperature.
2. Use aseptic technique when opening and dispensing reagents.
3. 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

Samples should be collected using trisodium citrate, acidified citrate or Stabilyte^{tm} (DiaPharma) collection media. Collection should be in accordance with the collection vials manufacturers instructions or in a 1:10 ratio of collection media to blood.

Immediately, upon collection of blood, the samples should be centrifuged at 3000 x g. This should ensure the removal of platelets as they can release PAI-1 that, in turn, complexes with uPA. The plasma can be transferred to a clean plastic tube and stored frozen for up to one month. Samples are stable for approximately 5 hours when stored at 4°C with the Sabilyte^{tm} media or 5 months if stored at -70°C.

Note: Detergents such as Triton X cause interference with the assay. If using detergent extracted samples, it is necessary to dialyze the samples overnight to remove the detergent.

REAGENT PREPARATION

The following solutions should be prepared fresh before starting the assay.

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 to 1x prior to use. Do this by combining 1 part of 10x Wash Buffer to 9 parts of DI Water relative to the amount required for the assay, either in whole or in part.

ASSAY PROCEDURE

1. Reconstitute both Standards and prepare the standard curve as indicated in the provided dilution table insert.

   Note: The Standards should be applied to the plate immediately upon preparation.
2. Add 80 µL of EIA Buffer to each well. Omit those wells intended for later use.
3. Add 20 µL of Standard or Unknown to the plate in duplicate wells. See Scheme I for a suggested template design.
4. Shake the plate at 300 rpm on a plate shaker for 30 minutes.
5. Wash the 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, then empty by inversion.
   e. Repeat step "c" two more times then proceed to step "f".
   f. Remove contents of the plate by inversion into an appropriate disposal device.
   g. Tap out remaining contents of the plate onto a lint free paper towel.

   Note: The decanted wells should be void of visible moisture before proceeding. If moisture is visible then follow step "b" until satisfactory results are obtained.
6. Add the indicated amount of 3% BSA Blocking Buffer directly to the Primary Antibody vial and slightly agitate until completely dissolved.
7. Add 100 µL of the reconstituted Primary Antibody Solution to the both standard and unknown test wells.
8. Shake plate at 300 rpm on the plate shaker for 30 minutes.
9. Wash wells according to step 6.
10. Combine the indicated amounts of the HRP Conjugated Secondary Antibody and 3% BSA Blocking Buffer and slightly agitate until completely dissolved.
11. Add 100 µL of the diluted Secondary Antibody solution to each well.
12. Shake plate at 300 rpm on the plate shaker for 30 minutes.
13. Wash wells according to step 6.
14. Add 100 µL of TMB Substrate to each well and shake plate for 5-10 minutes.
15. Stop the reaction with 50 µL per well of 1 N H_2SO_4 and read plate at 450 nm.
16. If accounting for substrate background, use 2 wells as blanks (BLK) with only the substrate in the wells (150 µL/well). Subtract the average of these absorbance values from the absorbance values of the wells being assayed.

Scheme I:

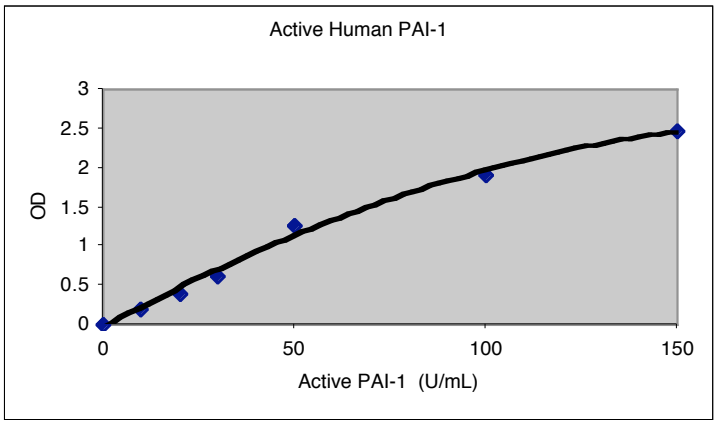
	1	2	3	4	5	6	7	8	9	10	11	12
A	S_0	S_0	U_1	U_1	U_9	U_9	U_{17}	U_{17}	U_{25}	U_{25}	U_{33}	U_{33}
B	S_1	S_1	U_2	U_2	U_{10}	U_{10}	U_{18}	U_{18}	U_{26}	U_{26}	U_{34}	U_{34}
C	S_2	S_2	U_3	U_3	U_{11}	U_{11}	U_{19}	U_{19}	U_{27}	U_{27}	U_{35}	U_{35}
D	S_3	S_3	U_4	U_4	U_{12}	U_{12}	U_{20}	U_{20}	U_{28}	U_{28}	U_{36}	U_{36}
E	S_4	S_4	U_5	U_5	U_{13}	U_{13}	U_{21}	U_{21}	U_{29}	U_{29}	U_{37}	U_{37}
F	S_5	S_5	U_6	U_6	U_{14}	U_{14}	U_{22}	U_{22}	U_{30}	U_{30}	U_{38}	U_{38}
G	S_6	S_6	U_7	U_7	U_{15}	U_{15}	U_{23}	U_{23}	U_{31}	U_{31}	U_{39}	U_{39}
H	S_7	S_7	U_8	U_8	U_{16}	U_{16}	U_{24}	U_{24}	U_{32}	U_{32}	BLK	BLK

CALCULATIONS

1. Subtract the average O.D. value of the blank wells (BLK) from all other pairs of wells. Some microplate readers can be programmed to do these subtractions automatically when reading the plate. Consult your instrument manual.
2. Average the O.D. values for each pair of duplicate wells.
3. Plot a standard curve using the average O.D. value for each standard value versus the concentration of standard.
4. Determine the concentration of each unknown by interpolation from the standard curve.

Conversion Factor: 1 PAI-1 unit = 1.34 ng

Typical Standard Curve:



PERFORMANCE CHARACTERISTICS

Assay Range: 0.0 - 112.5 U/mL (please refer to the lot specific standard)

Samples with uPA levels higher than the lot specific PAI-1 activity (~150 U/mL) should be diluted in similar media devoid of active uPA.

REFERENCES

1. Declerck, P. J., *et al*. J Intern Med (1994) 236:425-432
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