Colorimetric Assay for
Active Porcine 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 antiprotoelytic 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 functionally active PAI-1 is captured and quantitated with the use of an HRP labeled secondary antibody.

The PAI-1 binds to the PAI-1 capture antibody coated on the well of the microtiter plate. Next, a PAI-1 primary antibody is added to bind to the captured PAI-1 on the microtiter plate. An HRP conjugated secondary antibody is then added for detection of the total PAI-1. Optimal color is reached at 10 minutes when read at 450 nm. Quantitative test results are obtained by the measure and comparison of the sample and standard absorbance readings.

MATERIALS PROVIDED

Component	Contents	Volume	Storage	Cat. No.
Coated Plate	96-well microplate coated with uPA	1 plate	4°C	PI95a
Standard	Porcine PAI-1 activity standard; 50 ng/mL; lyophilized	1 vial	4°C	PI95b
Wash Buffer	10x solution for washing plate	50 mL	4°C	PI95c
Substrate	TMB Substrate	10 mL	4°C	PI95d
Primary Antibody	Anti-human PAI-1 monoclonal antibody; lyophilized	1 vial	4°C	PI95e
Secondary Antibody	Anti-mouse HRP conjugated antibody	10 mL	4°C	PI95f

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.

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™ (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 24 hours when stored at 4°C with the Sabilyte™ media.

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

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 the included 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.

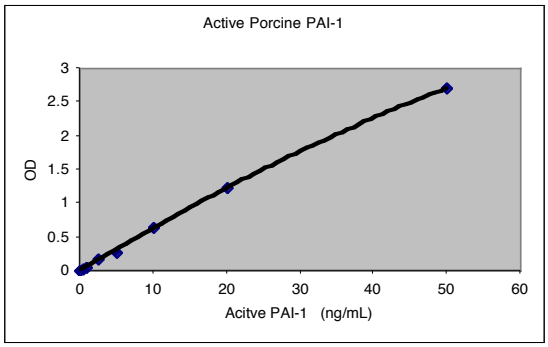
Scheme I:

	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	U_1	U_2
B	S_0	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	U_1	U_2
C	U_3	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}	U_{14}
D	U_3	U_4	U_5	U_6	U_7	U_8	U_9	U_{10}	U_{11}	U_{12}	U_{13}	U_{14}
E	U_{15}	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}	U_{26}
F	U_{15}	U_{16}	U_{17}	U_{18}	U_{19}	U_{20}	U_{21}	U_{22}	U_{23}	U_{24}	U_{25}	U_{26}
G	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}	U_{38}
H	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	U_{37}	U_{38}

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.

Typical Standard Curve:



PERFORMANCE CHARACTERISTICS

Assay Range: 0.0 - 50 ng/mL

Samples with uPA levels higher than 50 ng/mL should be diluted in similar media devoid of active uPA.

REFERENCES

1. Declerck, P., *et al*., (1995) Thromb Haemostas 74(5):1305-1309
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5. Schafer, K., *et al*., (2001) FASEB 15:1840-1842
6. Samad, F., *et al*., (1996) Proc Natl Acad Sci USA 96(12):6902-6907

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