Detection Assay for Total Mouse 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 all PAI-1 forms (complexed, latent and active) are captured and quantitated with the use of an HRP labeled secondary antibody.

The free, latent, or complexed 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	Volume	Storage
Anti-PAI-1 Coated Plate		4°C
Mouse PAI-1 Standard (50 ng/mL)	1 vial	4°C
10x Wash Buffer	50 mL	4°C
Anti-PAI-1 Primary Antibody	1 vial	4°C
TMB Substrate	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 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 or one month if stored at -20°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.

STANDARD PREPARATION

1. Reconstitute standard by adding 5 ml of blocking buffer directly to the vial and gently mix to dissolve the contents. This will provide a 50 ng/ml stock of PAI-1.
2. Perform a serial dilution as described in Table 1 below.

Table 1: Preparation of the Standard Curve

Standard	15-Isoprostane F_{2t} Concentration (ng/mL)	Blocking Buffer (µL)	Transfer Volume (µL)	Transfer Source	Final Volume (µL)
S_{10}	50	0	300	Standard Stock	300
S_9	20	600	400	Standard Stock	500
S_8	10	500	500	S_9	500
S_7	5	500	500	S_8	500
S_6	2.5	500	500	S_7	600
S_5	1	600	400	S_6	500
S_4	0.5	500	500	S_5	600
S_3	0.2	600	400	S_4	500
S_2	0.1	500	500	S_3	500
S_1	0.05	500	500	S_2	1,000
S_0	0	500	0	-	500

ASSAY PROCEDURE

1. Prepare standards as indicated in Table 1, above.
   Note: The standards should be applied to the plate immediately upon preparation.
2. Add 100 µL of standards or unknowns to each well omitting wells for later use. See **Scheme I** for suggested template design.
3. Shake the plate at 300 rpm on a plate shaker for 30 minutes.
4. 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. Repeat procedure 2 more times and proceed to step "f".
   f. Remove contents of the plate by inversion into an appropriate disposal device.
   g. Tap out the remaining contents of the plate onto a lint free paper towel and proceed to step 6.
   Note: The decanted wells should be void of visible moisture before proceeding. If moisture is still visible then follow step "g" until satisfactory results are obtained.
5. Make a working concentration of Primary Antibody by adding 10 ml of 3% BSA Blocking Buffer directly to the vial. Shake gently to dissolve the contents.
6. Add 100 µL of diluted Primary Antibody to each well.
7. Shake plate at 300 rpm on the plate shaker for 30 minutes.
8. Wash wells according to step 5 located above in this section.
9. Make a working concentration of Secondary Antibody. Briefly centrifuge the vial prior to opening. Add 1 ul of the secondary antibody to 10 ml of 3% BSA Blocking Buffer.
10. Add 100 µL of the working concentration Secondary Antibody solution to each well.
11. Shake plate at 300 rpm on the plate shaker for 30 minutes.

12. Wash wells according to step 5 located above in this section.
13. Add 100 µL of TMB substrate to each well and incubate for 10 minutes. If accounting for substrate background, use 2 wells as blanks with only substrate in the wells (150 µL/well).
14. Stop the reaction with 50 µL per well of 1 N H_2SO_4 and read plate at 450 nm.

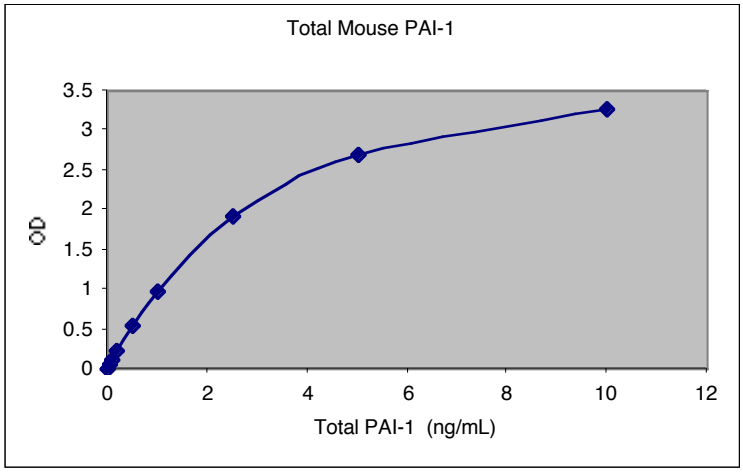
Scheme I:

	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}	BLK
H	U_{26}	U_{27}	U_{28}	U_{29}	U_{30}	U_{31}	U_{32}	U_{33}	U_{34}	U_{35}	U_{36}	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.
5. Note that low range standards (0-10 ng/ml) can be plotted linearly. If the entire range is desired, use software capable of 4-parameter logistic curve fitting.

Typical Standard Curve:



PERFORMANCE CHARACTERISTICS

Assay Range: 0.05-50 ng/mL

Samples with PAI-1 levels higher than 50 ng/mL should be diluted in similar media devoid of active PAI-1.

REFERENCES

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6. Samad, F., *et al*., (1996) Proc Natl Acad Sci USA 96(12):6902-6907

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