

## Activity Assay for Human PAI-1

For Research Use Only

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### INTRODUCTION

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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.

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### PRINCIPLES OF PROCEDURE

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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.

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### MATERIALS PROVIDED

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Component	Volume	Storage
uPA Coated Plate		4°C
Human PAI-1 Standard (0 U/mL)	2 vials	4°C
Human PAI-1 Standard (195 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

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### MATERIALS NEEDED BUT NOT PROVIDED

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- 1 N H<sub>2</sub>SO<sub>4</sub>
- TBS Buffer (see Reagent Preparation)
- Blocking Buffer (see Reagent Preparation)
- DI Water
- 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

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## STORAGE

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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.

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## WARNINGS AND PRECAUTIONS

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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.

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## PROCEDURAL NOTES

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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.

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## SAMPLE COLLECTION, STORAGE, AND PREPARATION

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Samples should be collected using trisodium citrate, acidified citrate, EDTA, or heparin 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 or 1 month if stored at frozen.

**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.

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## REAGENT PREPARATION

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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 w/v 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.

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**STANDARD PREPARATION**


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1. Reconstitute the 195 U standard and one vial of the 0 U standard by adding 1.0 mL of DI water to each and mixing gently. Standards should be used immediately and remaining standard should be frozen at -80°C for later use.

Standard	PAI-1 Concentration (U/mL)	0 U/mL PAI-1 Standard (μL)	Transfer Volume (μL)	Transfer Source	Final Volume (μL)
S <sub>10</sub>	100	47.5	50	195 U Stock	97.5
S <sub>9</sub>	50	72.5	25	195 U Stock	97.5
S <sub>8</sub>	25	85	12.5	195 U Stock	97.5
S <sub>7</sub>	10	92.5	5	195 U Stock	97.5
S <sub>6</sub>	5	180	5	195 U Stock	125
S <sub>5</sub>	2	90	60	S <sub>6</sub>	75
S <sub>4</sub>	1	75	75	S <sub>5</sub>	150
S <sub>3</sub>	0.5	75	75	S <sub>4</sub>	150
S <sub>2</sub>	0.25	75	75	S <sub>3</sub>	150
S <sub>1</sub>	0.125	75	75	S <sub>2</sub>	150
S <sub>0</sub>	0	75	0	---	75

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**ASSAY PROCEDURE**


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1. Add 80 μL of EIA Buffer to each well. Omit those wells intended for later use.
2. Add 20 μL of Standard or Unknown to the plate in duplicate wells. See Scheme I for a suggested template design.
3. Shake the plate at 300 rpm on a plate shaker for 30 minutes.
4. 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.
5. Add 11 mL of 3% BSA Blocking Buffer directly to the Primary Antibody vial and slightly agitate until completely dissolved.
6. Add 100 μL of the reconstituted Primary Antibody Solution to the both standard and unknown test wells.
7. Shake plate at 300 rpm on the plate shaker for 30 minutes.

8. Wash wells according to step 6.
9. Centrifuge the vial of HRP Conjugated Secondary Antibody and then add 1.0 uL to 10 mL of 3% BSA Blocking Buffer and slightly agitate until completely dissolved.
10. Add 100  $\mu$ L of the diluted 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 6.
13. Add 100  $\mu$ L of TMB Substrate to each well and shake plate for 2-10 minutes.
14. Stop the reaction with 50  $\mu$ L per well of 1 N H<sub>2</sub>SO<sub>4</sub> and read plate at 450 nm.
15. If accounting for substrate background, use 2 wells as blanks (BLK) with only the substrate in the wells (150  $\mu$ L/well). Subtract the average of these absorbance values from the absorbance values of the wells being assayed.

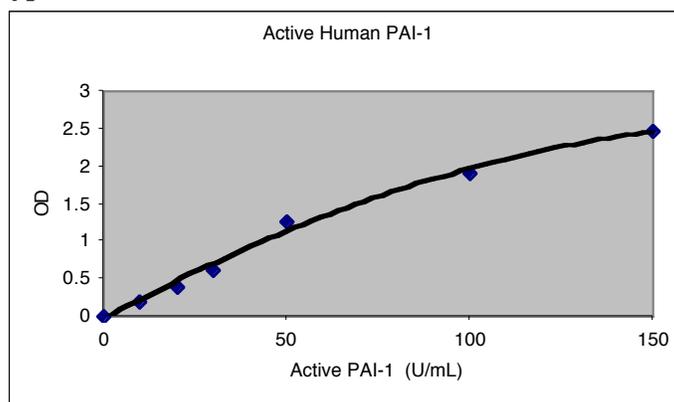
### Scheme I:

	1	2	3	4	5	6	7	8	9	10	11	12
A	S <sub>0</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	S <sub>6</sub>	S <sub>7</sub>	S <sub>8</sub>	S <sub>9</sub>	S <sub>10</sub>	U <sub>1</sub>
B	S <sub>0</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	S <sub>6</sub>	S <sub>7</sub>	S <sub>8</sub>	S <sub>9</sub>	S <sub>10</sub>	U <sub>1</sub>
C	U <sub>2</sub>	U <sub>3</sub>	U <sub>4</sub>	U <sub>5</sub>	U <sub>6</sub>	U <sub>7</sub>	U <sub>8</sub>	U <sub>9</sub>	U <sub>10</sub>	U <sub>11</sub>	U <sub>12</sub>	U <sub>13</sub>
D	U <sub>2</sub>	U <sub>3</sub>	U <sub>4</sub>	U <sub>5</sub>	U <sub>6</sub>	U <sub>7</sub>	U <sub>8</sub>	U <sub>9</sub>	U <sub>10</sub>	U <sub>11</sub>	U <sub>12</sub>	U <sub>13</sub>
E	U <sub>14</sub>	U <sub>15</sub>	U <sub>16</sub>	U <sub>17</sub>	U <sub>18</sub>	U <sub>19</sub>	U <sub>20</sub>	U <sub>21</sub>	U <sub>22</sub>	U <sub>23</sub>	U <sub>24</sub>	U <sub>25</sub>
F	U <sub>14</sub>	U <sub>15</sub>	U <sub>16</sub>	U <sub>17</sub>	U <sub>18</sub>	U <sub>19</sub>	U <sub>20</sub>	U <sub>21</sub>	U <sub>22</sub>	U <sub>23</sub>	U <sub>24</sub>	U <sub>25</sub>
G	U <sub>26</sub>	U <sub>27</sub>	U <sub>28</sub>	U <sub>29</sub>	U <sub>30</sub>	U <sub>31</sub>	U <sub>32</sub>	U <sub>33</sub>	U <sub>34</sub>	U <sub>35</sub>	U <sub>36</sub>	BLK
H	U <sub>26</sub>	U <sub>27</sub>	U <sub>28</sub>	U <sub>29</sub>	U <sub>30</sub>	U <sub>31</sub>	U <sub>32</sub>	U <sub>33</sub>	U <sub>34</sub>	U <sub>35</sub>	U <sub>36</sub>	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.

### Typical Standard Curve:



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**PERFORMANCE CHARACTERISTICS**

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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.

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**REFERENCES**

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**TECHNICAL SUPPORT**

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If you need technical information or assistance with assay procedures, call our Technical Support Department at 1-800-692-4633 or 1-248-852-8815. Our staff will be happy to answer your questions about this or any other product in the Oxford Biomedical line.

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