ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Human PAI-1 ELISA Kit

(Catalog Number: 31070)

For the quantitative determination of human PAI-1 concentrations in serum or plasma

IMD (Hong Kong)
Address: Unit 513, 5/F, Biotech Centre 2, No. 11 Science Park West Avenue, Hong Kong Science Park, Sha Tin, Hong Kong
Website: www.immunodiagnostics.com.hk
Email: info@immunodiagnostics.com.hk
Tel: (+852) 3502 2780

IMD (Canada)
Address: 3330 Bayview Avenue, Block #6, Toronto, M2M 3R8, Ontario, Canada
Email: info@immunodiagnostics.ca
Tel: +1-437-886-5136

Version 5.0

TABLE OF CONTENTS

INTRODUCTION

Plasminogen activator inhibitor-1 (PAI-1) is the primary inhibitor of tissue-type and urokinase-type plasminogen activator, playing a major role in fibrinolysis[1,2]. PAI-1 is mainly produced by the endothelium, but is also secreted by other tissue types, such as adipose tissue[3]. It is normally present at low levels in plasma and tissue, but its expression and release are increased in various disease states (such as a number of forms of cancer), as well as in obesity and the metabolic syndrome[4]. PAI-1 is also involved in the pathophysiology of renal, pulmonary, cardiovascular, and metabolic diseases[5-8]. Elevated local or systemic PAI-1 can also exacerbate such pathologic conditions.

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). The microtiter plate is precoated with a mouse monoclonal antibody specific for human PAI-1. Standards and samples are pipetted into the wells and any human PAI-1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin labelled polyclonal antibody specific for human PAI-1 is added to the wells. After wash step to remove any unbound reagents, streptavidin-horseradish peroxidase conjugate (STP-HRP) is added. After the last wash step, an HRP substrate solution 3,3′,5,5′-Tetramethylbenzidine (TMB) is added and color develops in proportion to the amount of human PAI-1 bound initially. The assay is stopped, and the optical density of the wells is determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured human PAI-1, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This Human PAI-1 ELISA kit is designed for quantification of human PAI-1 in serum and plasma samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Microtiter Strips (96 wells), coated with a mouse monoclonal antibody against human PAI-1, sealed
2. 10×Wash buffer, 50 mL
3. 5×Assay buffer, 20 mL
4. 100×Detection antibody solution, a biotin labelled polyclonal antibody against human PAI-1, 0.12 mL
5. Human PAI-1 standard, 2 ng of recombinant human PAI-1, lyophilized
6. 200×STP-HRP solution, 0.06 mL
7. Substrate solution, 12 mL, ready for use
8. Stop solution, 12 mL, ready for use

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips
2. 96-well plate or manual strip washer

3. Buffer and reagent reservoirs
4. Paper towels or absorbent paper
5. Plate reader capable of reading absorbance at 450 nm
6. Distilled water or deionized water

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the Human PAI-1 microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer

Prepare 1×Assay buffer by mixing the 5×Assay buffer (20 mL) with 80 mL of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer

Prepare 1×Wash buffer by mixing the 10×Wash buffer (50 mL) with 450 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1×Detection antibody solution

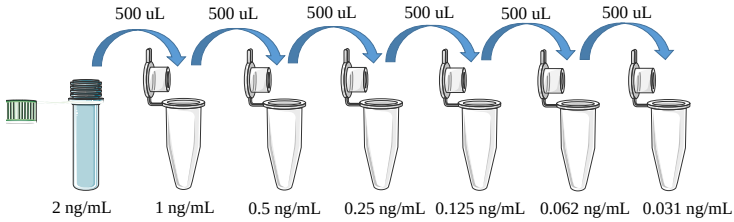
Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

D. 1×STP-HRP solution

Spin down the 200×STP-HRP solution briefly and dilute the desired amount of the 200×STP-HRP solution 1:200 with 1×Assay buffer, 100 µL of the 1×STP-HRP solution is required per well. Prepare only as much 1×STP-HRP solution as needed. Return the 200×STP-HRP solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDARDS AND SAMPLES

Human PAI-1 Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer to generate a standard stock solution of 2 ng/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Pipette 500 µL of 1×Assay buffer to 1, 0.5, 0.25, 0.125, 0.062, 0.031 ng/mL tubes. Use the standard stock solution to produce a serial dilution as shown below.



1×Assay buffer serves as the zero standard (0 ng/mL). The reconstituted standard stock should be aliquoted and stored at -20ºC for one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample Preparation:

Serum samples generally require about **5-fold** dilution in this assay. A suggested dilution step is to add 20 µL of sample to 80 µL of 1×Assay buffer. Different plasma preparations (EDTA, heparin or citrate) may result in different concentration of human PAI-1. It is recommended that the users establish their own dilution factors based on their samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 100 µL of standard or sample per well, incubate at room temperature for 1 hour.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 µL of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 3 times as in step 2.
5. Add 100 µL of 1×STP-HRP solution to each well, incubate at room temperature for 20 minutes.
6. Wash each well 4 times as described in step 2.
7. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
8. Add 100 µL of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
9. Measure absorbance of each well at 450 nm immediately.

CALCULATION

1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human PAI-1 concentrations (x-axis). The best fit line can be generated with any

curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.

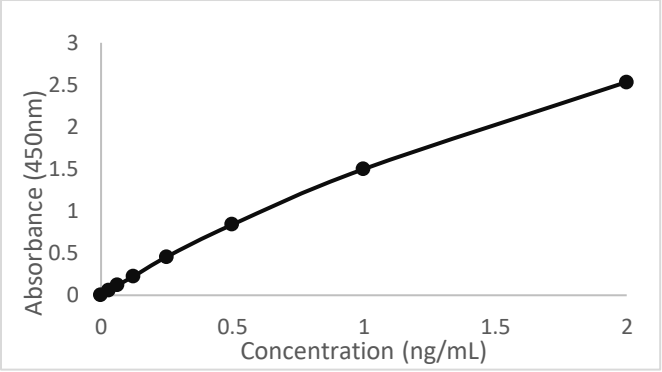
3. Determine human PAI-1 concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay.

PAI-1 (ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.098	0
0.031	0.158	0.06
0.062	0.217	0.119
0.125	0.325	0.227
0.25	0.555	0.457
0.5	0.938	0.84
1.0	1.598	1.5
2.0	2.632	2.534

Human PAI-1 standard curve



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of PAI-1 that can be measured by this assay is 0.031 ng/mL.

B. Specificity

The antibodies used in this assay are specific to human PAI-1 and do not cross-react with mouse and rat PAI-1, and other cytokine or hormone molecules including human resistin, TNFα, ANGPTL4, insulin, leptin and IL6.

C. Precision

Intra-assay Precision (Precision within an assay)

Two samples of known concentration were tested 16 times on one plate.

Sample	Mean (ng/mL)	SD (ng/mL)	C.V. (%)
1	3.93	0.129	3.31
2	5.14	0.096	1.87

Inter-assay Precision (Precision between assays)

Four samples of known concentration were tested in 8 separate assays.

Sample	Mean (ng/mL)	SD (ng/mL)	C.V. (%)
1	0.64	0.029	4.60
2	1.26	0.053	4.23
3	2.44	0.065	2.69
4	5.06	0.188	3.73

D. Recovery

Serum samples were spiked with different amounts of human PAI-1 and assayed.

Sample	Average % Recovery	Range %
Serum (n=4)	99%	88-114

REFERENCES

1. Lijnen HR, et al. (1995) *Baillieres Clin Haematol*. 8: 277–290
2. Dellas C, et al. (2005) *Thromb Haemost.* 2005; 93: 631–640
3. Binder BR, et al. (2002) *News Physiol Sci* 17:56-61
4. Vague P, et al. (1986) *Metabolism.* 35: 250–253
5. Sobel Be, et al. (2003) *Arterioscler Thromb Vasc Biol.* 23: 1979–1989
6. Eitzman DT, et al. (1996) *J Clin Invest.* 97(1):232-7
7. Gils A, et al. (2004) *Thromb. Haemost.* 91 (3): 425–37
8. Cale JM, Lawrence DA. (2007) *Curr Drug Targets*. 8(9):971-81

SUMMARY OF ASSAY PROCEDURE

Add 100 µL of Standard or sample per well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1×Detection antibody solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1×STP-HRP solution to each well.
↓
Incubate at room temperature for 20 minutes.
↓
Aspirate and wash each well four times.
↓
Add 100 µL of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µL of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation