BioVendor Group

ENG

Instructions for Use:

HUMAN PAI-1 ELISA

Catalogue number:

RAF083R

For research use only!

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current version
ENG.006.A	ENG.007.A
History of changes added.	
A symbol indicating the manufacturer added.	

1. INTENDED USE

The Human PAI-1 ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human PAI-1. **The Human PAI-1 ELISA is for research use only. Not for use in diagnostic procedures.**

2. SUMMARY

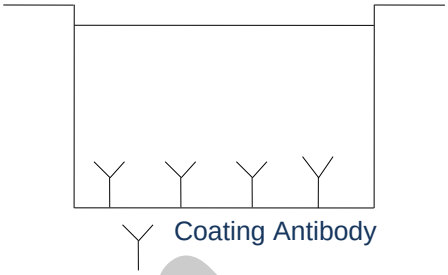
Plasminogen activator inhibitor-1 (PAI-1) is the primary inhibitor of plasminogen activators in plasma, rapidly inactivating both tissue plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). PAI-1 is a single-chain glycoprotein with a molecular weight of 47 kilodaltons. During fibrinolysis, tissue plasminogen activator (tPA) converts the inactive protein plasminogen into plasmin which plays a critical role in fibrinolysis by degrading fibrin and providing localized protease activity in a number of physiological functions. PAI-1 is synthesized in the liver and by endothelial cells, and its synthesis is regulated by several physiologic mediators, including endotoxin, interleukin-1, fibroblast growth factor-2, and lipids. Plasminogen activator inhibitor-1 is an important inhibitor of the fibrinolytic system, so elevated levels could suppress fibrinolysis and result in an increased risk of thrombosis. Increased PAI-1 levels have been shown to be associated with a number of atherosclerotic risk factors, PAI-1 has been shown to act as a prothrombic factor in both arterial and venous thromboembolic disorders.

3. PRINCIPLES OF THE TEST

An anti-human PAI-1 coating antibody is adsorbed onto microwells.

Figure 1

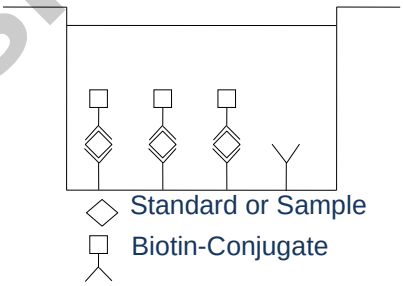
Coated Microwell



Human PAI-1 present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated anti-human PAI-1 antibody is added and binds to human PAI-1 captured by the first antibody.

Figure 2

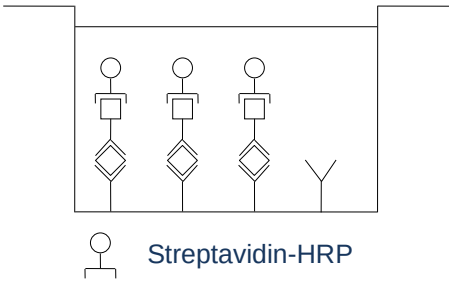
First Incubation



Following incubation unbound biotin-conjugated anti-human PAI-1 antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human PAI-1 antibody.

Figure 3

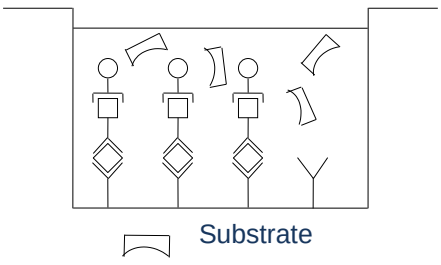
Second Incubation



Following incubation unbound Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

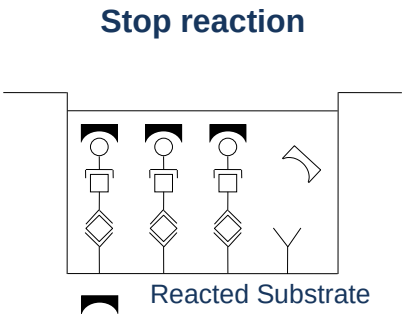
Figure 4

Third Incubation



A colored product is formed in proportion to the amount of human PAI-1 present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human PAI-1 standard dilutions and human PAI-1 sample concentration determined.

Figure 5



4. REAGENTS PROVIDED

1 aluminium pouch with an **Antibody Coated Microtiter Strips** (12 strips with 8 wells each) coated with monoclonal antibody to human PAI-1
1 vial (70 µl) **Biotin-Conjugate** anti-human PAI-1 polyclonal antibody
1 vial (150 µl) **Streptavidin-HRP**
2 vials human PAI-1 **Standard** lyophilized, 10,000 pg/ml upon reconstitution
1 vial (5 ml) **Assay Buffer Concentrate** 20x (PBS with 1% Tween 20 and 10% BSA)
1 bottle (50 ml) **Wash Buffer Concentrate** 20x (PBS with 1% Tween 20)
1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)
1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)
4 **Adhesive Films**

5. STORAGE INSTRUCTIONS – ELISA KIT

Store kit reagents between 2°C and 8°C. Immediately after use remaining reagents should be returned to cold storage (2°C to 8°C). Expiry of the kit and reagents is stated on labels.
Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

6. SAMPLE COLLECTION AND STORAGE INSTRUCTIONS

Cell culture supernatant, serum and plasma (EDTA, citrate, heparinized) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Pay attention to a possible “Hook Effect” due to high sample concentrations (see chapter Calculation of results).

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic samples.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive human PAI-1. If samples are to be run within 24 hours, they may be stored at 2° to 8°C
(for sample stability refer to 13.5).

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

7. MATERIALS REQUIRED BUT NOT PROVIDED

- 5 ml and 10 ml graduated pipettes
- 5 µl to 1000 µl adjustable single channel micropipettes with disposable tips
- 50 µl to 300 µl adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

8. PRECAUTIONS FOR USE

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Exposure to acid inactivates the conjugate.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose samples and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9. PREPARATION OF REAGENTS

– Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure.
– If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved

9.1 Wash Buffer (1x)

1. Pour entire contents (50 ml) of the Wash Buffer Concentrate (20x) into a clean 1000 ml graduated cylinder. Bring to final volume of 1000 ml with glass-distilled or deionized water. Mix gently to avoid foaming.
2. Transfer to a clean wash bottle and store at 2° to 25°C.
3. The Wash Buffer (1x) is stable for 30 days.
4. Wash Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20x) (ml)	Distilled Water (ml)
1 - 6	25	475
1 - 12	50	950

9.2 Assay Buffer (1x)

1. Pour the entire contents (5 ml) of the Assay Buffer Concentrate (20x) into a clean 100 ml graduated cylinder. Bring to final volume of 100 ml with distilled water. Mix gently to avoid foaming.
2. Store at 2°C to 8°C. Please note that the Assay Buffer (1x) is stable for 30 days.
3. Assay Buffer (1x) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

9.3 Biotin-Conjugate

Note: The Biotin-Conjugate should be used within 30 minutes after dilution.
Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (1x) (ml)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

9.4 Streptavidin-HRP

Note: The Streptavidin-HRP should be used within 30 minutes after dilution.
Make a 1:200 dilution of the concentrated Streptavidin-HRP solution with Assay Buffer (1x) in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (1x) (ml)
1 - 6	0.03	5.97
1 - 12	0.06	11.94

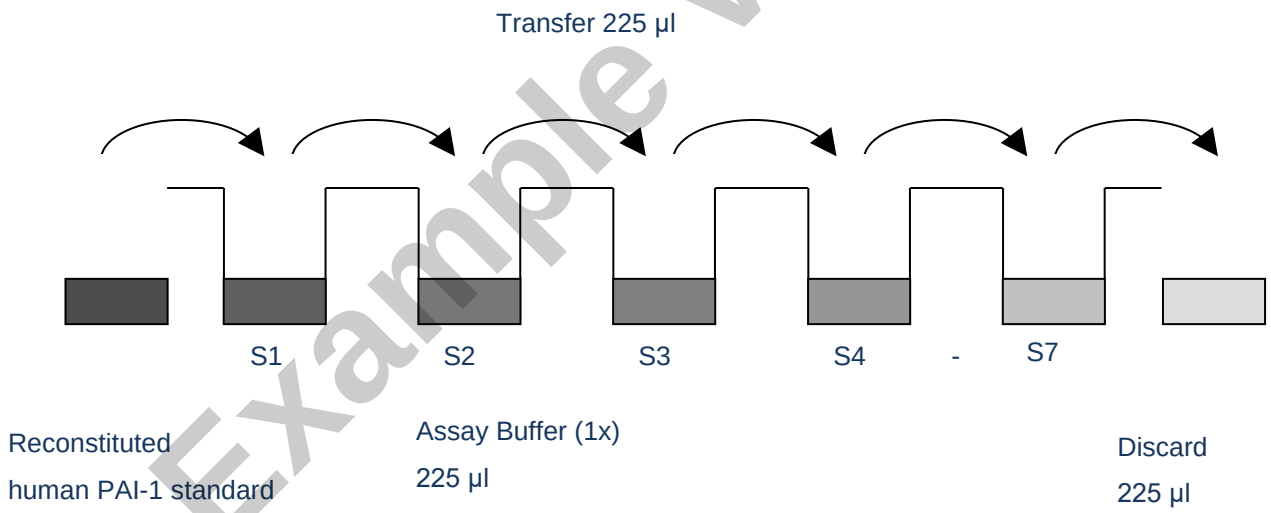
9.5 Human PAI-1 Standard

1. Reconstitute human PAI-1 standard by addition of distilled water.
2. Refer to the Quality Control Sheet for current volume of Distilled water needed for reconstitution of standard. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 10,000 pg/ml). Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.
3. The standard has to be used immediately after reconstitution and cannot be stored.

9.5.1 External standard dilution

1. Label 7 tubes, one for each standard point: S1, S2, S3, S4, S5, S6, S7.
2. Prepare 1:2 serial dilutions for the standard curve as follows: Pipette 225 µl of Assay Buffer (1x) into each tube.
3. Pipette 225 µl of reconstituted standard (concentration = 10,000 pg/ml) into the first tube, labelled S1, and mix (concentration of standard 1 = 5000 pg/ml).
4. Pipette 225 µl of this dilution into the second tube, labelled S2 , and mix thoroughly before the next transfer.
5. Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 6). Assay Buffer (1x) serves as blank.

Figure 6 Dilute standards – tubes



10. TEST PROTOCOL

Note: In case of incubation without shaking the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

1. Predilute your samples before starting with the test procedure. Dilute samples 1:50 with Assay Buffer (1x) according to the following scheme:
   Dilution: 10 µl sample + 490 µl Assay Buffer (1x)
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank and optional control sample should be assayed in duplicate. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2° to 8°C sealed tightly.
3. Wash the microwell strips twice with approximately 400 µl Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10 - 15 seconds before aspiration. Take care not to scratch the surface of the microwells.
   After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.
4. Standard dilution on the microwell plate (alternatively the standard dilution can be prepared in tubes):
   Add 100 µl Assay Buffer (1x) in duplicate to all standard wells. Pipette 100 µl of prepared standard (see Preparation of Standard, concentration = 10,000 pg/ml) in duplicate into well A1 and A2 (see Table 1). Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 5000 pg/ml), and transfer 100 µl to wells B1 and B2, respectively (see Figure 7) Take care not to scratch the inner surface of the microwells. Continue this procedure 5 times, creating two rows of human PAI-1 standard dilutions ranging from 5000-78 pg/ml. Discard 100 µl of the contents from the last microwells (G1, G2) used.

Figure 7 Dilute standards – microwell plate

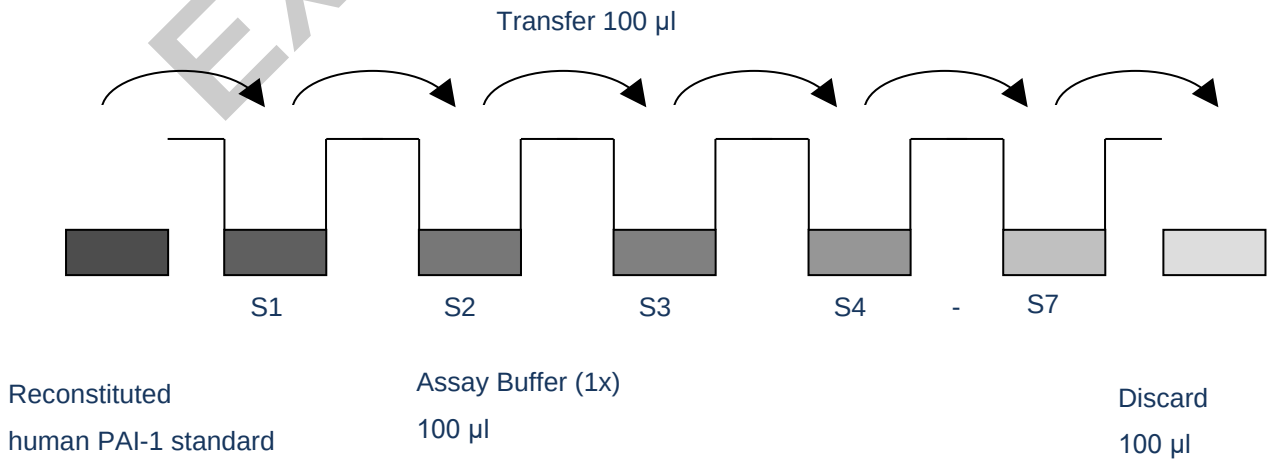


Table 1 Example of the arrangement of blanks, standards and samples in the microwell strips.

	1	2	3	4
A	Standard 1 (5000 pg/ml)	Standard 1(5000 pg/ml)	Sample 1	Sample 1
B	Standard 2 (2500 pg/ml)	Standard 2 (2500 pg/ml)	Sample 2	Sample 2
C	Standard 3 (1250 pg/ml)	Standard 3 (1250 pg/ml)	Sample 3	Sample 3
D	Standard 4 (625 pg/ml)	Standard 4 (625 pg/ml)	Sample 4	Sample 4
E	Standard 5 (313 pg/ml)	Standard 5 (313 pg/ml)	Sample 5	Sample 5
F	Standard 6 (156 pg/ml)	Standard 6 (156 pg/ml)	Sample 6	Sample 6
G	Standard 7 (78 pg/ml)	Standard 7 (78 pg/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

In case of an external standard dilution (see External standard dilution 9.5.1), pipette 100 µl of these standard dilutions (S1 -S7) in the standard wells according to Table 1

5. Add 100 µl of Assay Buffer (1x) in duplicate to the blank wells.
6. Add 50 µl of Assay Buffer (1x) to the sample wells.
7. Add 50 µl of prediluted sample in duplicate to the sample wells.
8. Prepare Biotin-Conjugate (see Biotin-Conjugate 9.3).
9. Add 50 µl of Biotin-Conjugate to all wells.
10. Cover with an adhesive film and incubate at room temperature (18 to 25°C) for 2 hours on a microplate shaker.
11. Prepare Streptavidin-HRP (see Streptavidin-HRP 9.4).
12. Remove adhesive film and empty wells. Wash microwell strips 3 times according to step 3. Proceed immediately to the next step.
13. Add 100 µl of diluted Streptavidin-HRP to all wells, including the blank wells.
14. Cover with an adhesive film and incubate at room temperature (18° to 25°C) for 1 hour on a microplate shaker.
15. Remove adhesive film and empty wells. Wash microwell strips 3 times according to step 3. Proceed immediately to the next step.
16. Pipette 100 µl of TMB Substrate Solution to all wells.
17. Incubate the microwell strips at room temperature (18° to 25°C) for about 10 minutes. Avoid direct exposure to intense light.
    The colour development on the plate should be monitored and the substrate reaction stopped (see next step) before positive wells are no longer properly recordable. Determination of the ideal time period for colour development has to be done individually for each assay**.** It is recommended to add the Stop Solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.
18. Stop the enzyme reaction by quickly pipetting 100 µl of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

19. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

11. CALCULATION OF RESULTS

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 % of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human PAI-1 concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human PAI-1 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human PAI-1 concentration.
- If instructions in this protocol have been followed samples have been diluted 1:100 (50 µl 1:50 prediluted sample + 50 µl Assay Buffer (1x)), the concentration read from the standard curve must be multiplied by the dilution factor (x 100).
- Calculation of samples with a concentration exceeding standard 1 will result in incorrect, low human PAI-1 levels (Hook Effect).
- Such samples require further external predilution according to expected human PAI-1 values with Assay Buffer (1x) in order to precisely quantitate the actual human PAI-1 level.
- It is suggested that each testing facility establishes a control sample of known human PAI-1 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 8

Note: Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

Figure 7 Representative standard curve for Human PAI-1 ELISA kit. Human PAI-1 was diluted in serial 2-fold steps in Assay Buffer (1x)

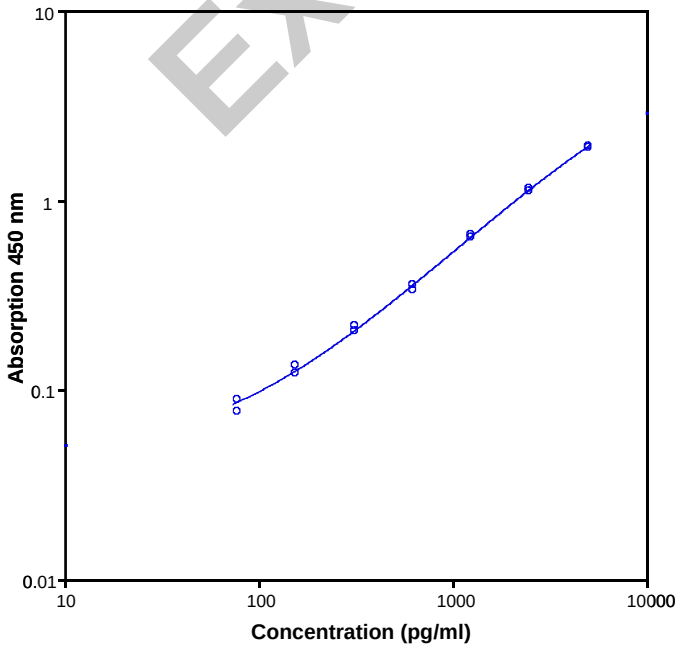


Table 2 Typical data using the Human PAI-1 ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Human PAI-1 Concentration (pg/ml)	O.D. at 450 nm	Mean O.D. at 450 nm	C.V. (%)
1	5000	1.934	1.910	1.3
		1.886		
2	2500	1.114	1.133	1.7
		1.152		
3	1250	0.632	0.642	1.6
		0.652		
4	625	0.337	0.347	3.0
		0.357		
5	313	0.218	0.211	3.7
		0.203		
6	156	0.134	0.127	5.0
		0.121		
7	78	0.088	0.083	6.4
		0.077		
Blank	0	0.037	0.038	1.2
		0.038		

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

12. LIMITATIONS

- Because exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA).

HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results.
Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

13. PERFORMANCE CHARACTERISTICS

13.1 Sensitivity

The limit of detection of human PAI-1 defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 29.0 pg/ml (mean of 6 independent assays).

13.2 Reproducibility

13.2.1 Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human PAI-1. Two standard curves were run on each plate. Data below show the mean human PAI-1 concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 4.7%.

Table 3 The mean human PAI-1 concentration and the coefficient of variation for each sample

Sample	Experiment	Mean human PAI-1 Concentration (ng/ml)	Coefficient of Variation (%)
1	1	53.7	5.9
	2	60.1	1.9
	3	57.2	1.7
2	1	7.7	11.6
	2	8.3	3.2
	3	8.7	6.0
3	1	133.8	5.1
	2	139.4	1.9
	3	128.5	5.7
4	1	62.0	4.2
	2	59.4	2.6
	3	55.6	3.7
5	1	44.0	6.4
	2	43.3	4.5
	3	40.6	7.2
6	1	28.4	5.5
	2	33.6	2.9
	3	30.3	4.2
7	1	31.5	6.0
	2	33.5	1.6
	3	30.5	4.0
8	1	51.4	9.4

Sample	Experiment	Mean human PAI-1 Concentration (ng/ml)	Coefficient of Variation (%)
	2	52.5	1.4
	3	51.4	4.9

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 8 serum samples containing different concentrations of human PAI-1. Two standard curves were run on each plate. Data below show the mean human PAI-1 concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 5.0%.

Table 4 The mean human PAI-1 concentration and the coefficient of variation of each sample

Sample	Mean human PAI-1 concentration (ng/ml)	Coefficient of variation (%)
1	57.0	5.6
2	8.2	5.8
3	133.9	4.1
4	59.0	5.5
5	42.6	4.2
6	30.8	8.5
7	31.8	4.8
8	51.7	1.2

13.3 Spiking recovery

The spiking recovery was evaluated by spiking 4 levels of human PAI-1 into serum and plasma. Recoveries were determined in 3 independent experiments with 4 replicates each.
The amount of endogenous human PAI-1 in unspiked samples was subtracted from the spike values.
The recovery ranged from 64.5 - 84.8% with an overall mean recovery of 72.2% for serum and from 44.8 - 68.4% with an overall mean recovery of 58.7% for plasma samples.

13.4 Dilution linearity

Serum samples with different levels of human PAI-1 were analysed at serial 2 fold dilutions with 4 replicates each.
The recovery ranged from 91.9 - 111.8% with an overall recovery of 98.7% (see Table 5).

Table 5

Sample	Dilution	Expected human PAI-1 concentration (ng/ml)	Observed human PAI-1 concentration (ng/ml)	Recovery of expected human PAI-1 concentration (%)
1	1	-	164.9	-
	2	82.4	78.6	95.3
	4	41.2	39.3	95.3
	8	20.6	19.5	94.4
2	1	-	127.5	-
	2	63.7	63.0	98.8
	4	31.9	30.0	94.2
	8	15.9	16.5	103.5
3	1	-	60.4	-
	2	30.2	28.5	94.5
	4	15.1	14.6	96.8
	8	7.6	8.2	108.8
4	1	-	46.9	-
	2	23.5	21.5	91.9
	4	11.7	11.6	98.8
	8	5.9	6.6	111.8

13.5 Sample stability

13.5.1 Freeze-thaw stability

Aliquots of serum and plasma samples (spiked or unspiked) were stored at -20°C and thawed 5 times, and the human PAI-1 levels determined. There was no significant loss of human PAI-1 immunoreactivity detected by freezing and thawing.

13.5.2 Storage stability

Aliquots of serum and plasma samples (spiked or unspiked) were stored at -20°C, 2-8°C and room temperature (RT), and the human PAI-1 level determined after 24 h. There was no significant loss of human PAI-1 immunoreactivity detected during storage under above conditions.

13.6 Specificity

The assay detects both natural and recombinant human PAI-1. The interference of circulating factors of the immune systeme was evaluated by spiking these proteins at physiologically relevant concentrations into serum. No cross-reactivity was detected.

13.7 Expected values

A panel of serum and plasma samples from randomly selected apparently healthy donors (males and females) was tested for human PAI-1.
The detected human PAI-1 levels for sera ranged between 1.2 and 286.0 ng/ml with a mean level of 41.9 ng/ml for plasma (EDTA) between 0.0 and 110.0 ng/ml with a mean level of 14.1 ng/ml, for plasma (heparinized) between 0.9 and 99.0 ng/ml with a mean level of 22.9 ng/ml and for plasma (citrate) between 2.4 and 43.8 ng/ml with a mean level of 17.9 ng/ml.

14. REAGENT PREPARATION SUMMARY

14.1 Wash Buffer

Add Wash Buffer Concentrate 20x (50 ml) to 950 ml distilled water.

Number of Strips	Wash Buffer Concentrate (ml)	Distilled Water (ml)
1 – 6	25	475
1 – 12	50	950

14.2 Assay Buffer (1x)

Add Assay Buffer Concentrate 20x (5 ml) to 95 ml distilled water.

Number of Strips	Assay Buffer Concentrate (ml)	Distilled Water (ml)
1 – 6	2.5	47.5
1 – 12	5.0	95.0

14.3 Biotin-Conjugate

Make a 1:100 dilution of Biotin-Conjugate in Assay Buffer (1x):

Number of Strips	Biotin-Conjugate (ml)	Assay Buffer (1x) (ml)
1 – 6	0.03	2.97
1 – 12	0.06	5.94

14.4 Streptavidin-HRP

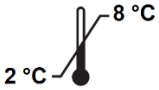
Make a 1:200 dilution of Streptavidin-HRP in Assay Buffer (1x):

Number of Strips	Streptavidin-HRP (ml)	Assay Buffer (1x) (ml)
1 – 6	0.03	5.97
1 – 12	0.06	11.94

14.5 Human PAI-1 Standard

Reconstitute lyophilized human PAI-1 standard with distilled water. (Reconstitution volume is stated in the Quality Control Sheet.)

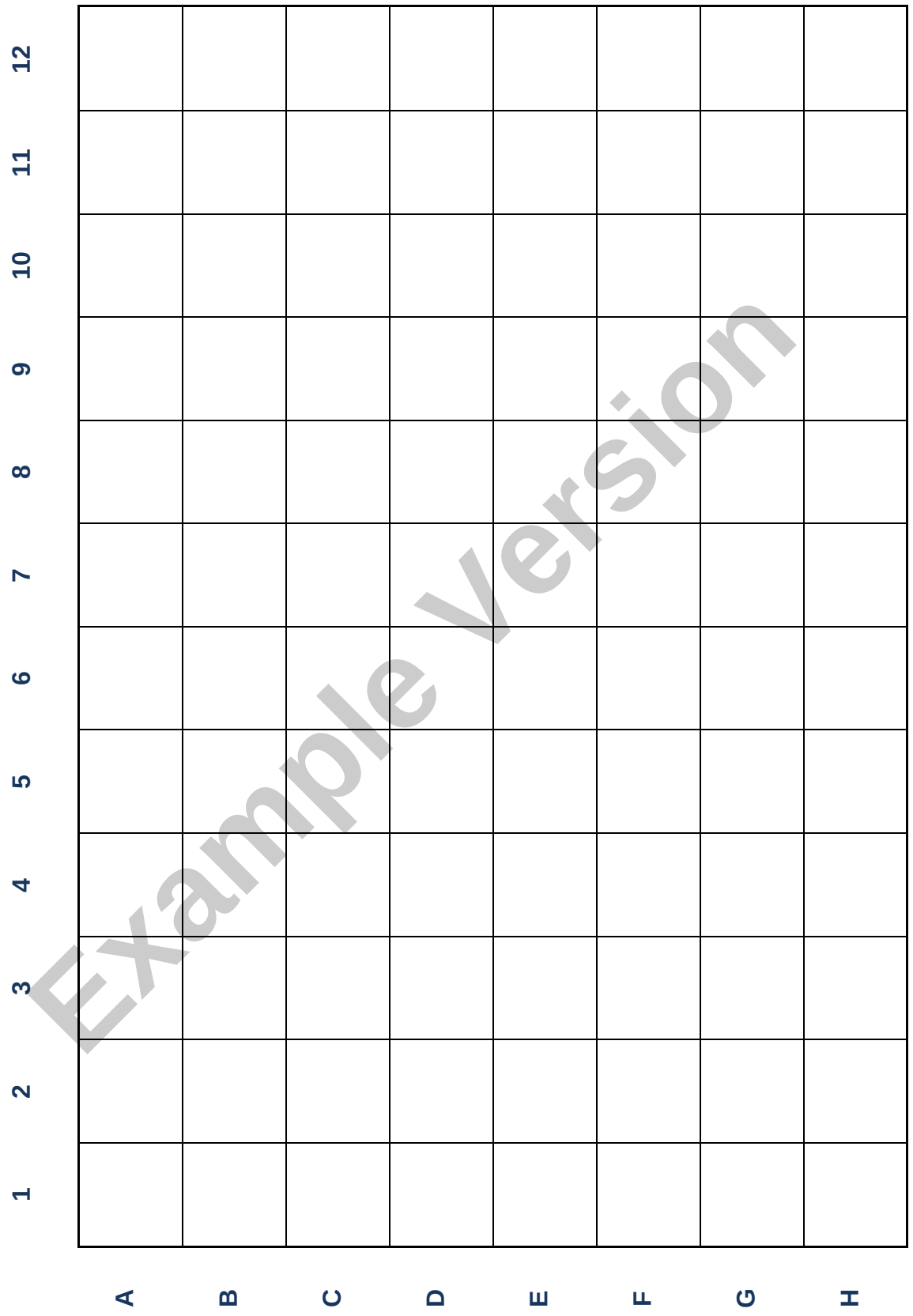
15. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks

16. ASSAY PROCEDURE - SUMMARY

Note: If instructions in this protocol have been followed samples have been diluted 1:100 (50 µl 1:50 prediluted sample + 50 µl Assay Buffer (1x)), the concentration read from the standard curve must be multiplied by the dilution factor (x 100).

1. Predilute sample 1:50 with Assay Buffer (1x)
2. Determine the number of microwell strips required.
3. Wash microwell strips twice with Wash Buffer.
4. Standard dilution on the microwell plate: Add 100 µl Assay Buffer (1x), in duplicate, to all standard wells. Pipette 100 µl prepared standard into the first wells and create standard dilutions by transferring 100 µl from well to well. Discard 100 µl from the last wells.
   Alternatively, external standard dilution in tubes (see page 9): Pipette 100 µl of these standard dilutions in the microwell strips.
5. Add 100 µl Assay Buffer (1x), in duplicate, to the blank wells.
6. Add 50 µl Assay Buffer (1x) to sample wells.
7. Add 50 µl prediluted sample in duplicate, to designated sample wells.
8. Prepare Biotin-Conjugate.
9. Add 50 µl Biotin-Conjugate to all wells.
10. Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C).
11. Prepare Streptavidin-HRP.
12. Empty and wash microwell strips 3 times with Wash Buffer.
13. Add 100 µl diluted Streptavidin-HRP to all wells.
14. Cover microwell strips and incubate 1 hour at room temperature (18º to 25ºC).
15. Empty and wash microwell strips 3 times with Wash Buffer.
16. Add 100 µl of TMB Substrate Solution to all wells.
17. Incubate the microwell strips for about 10 minutes at room temperature (18°to 25°C).
18. Add 100 µl Stop Solution to all wells.
19. Blank microwell reader and measure colour intensity at 450 nm.



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