ab157528 – Human PAI1 ELISA Kit

Instructions for Use

For the quantitative determination of active plasminogen activator inhibitor type 1 in Human plasma.

This product is for research use only and is not intended for diagnostic use.

Version 10, Last Updated August 3, 2022

Table of Contents

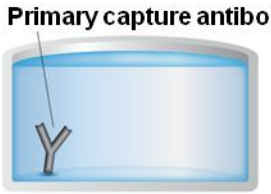
1. BACKGROUND

Abcam's Human PAI1 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for is for the quantitative determination of active plasminogen activator inhibitor type 1 in Human plasma.

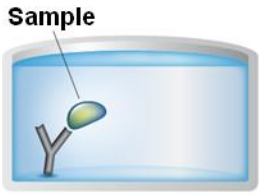
PAI1 is involved in the regulation of the blood fibrinolytic system. Increased plasma levels of PAI1 are implicated in the impairment of fibrinolytic function and may be associated with thrombotic diseases. Levels of PAI1 increase with age and are elevated in conditions such as normal pregnancy and sepsis.

Functionally active PAI1 present in plasma reacts with urokinase coated and dried on a microtiter plate. Latent or complexed PAI1 will not bind to the plate or be detected. Unbound PAI1 samples are aspirated and an anti-PAI1 primary antibody is added. Excess primary antibody is then aspirated. The bound antibody, which is proportional to the original active PAI1 present in the samples, is then reacted with the horseradish peroxidase conjugated secondary antibody. Following an additional washing step, TMB substrate solution is then used for color development at 450nm. The amount of color development is directly proportional to the concentration of active PAI1 in the sample.

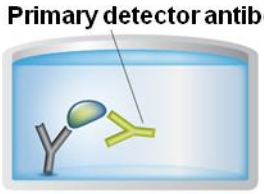
2. ASSAY SUMMARY



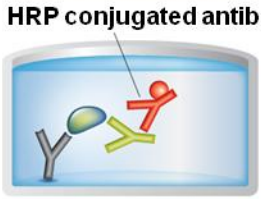
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.



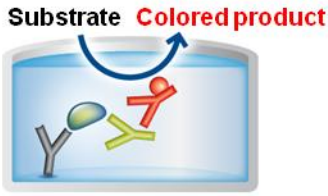
Add standard or sample to each well used. Incubate at room temperature.



Aspirate and wash each well. Add primary detector antibody. Incubate at room temperature.



Aspirate and wash each well. Add HRP conjugated antibody to each well. Incubate at room temperature.



Aspirate and wash each well. Add TMB Substrate to each well. Immediately begin recording the color development

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.
- The PAI1 activity standards are of Human origin. Each donor unit has been tested and found negative for the presence of HBsAg, anti-HIV 1+2, anti-HBc, and anti-HCV. Since no tests are currently available to assure that no infectious agents are present, the plasma must be treated as potentially hazardous.

4. STORAGE AND STABILITY

Store kit at 2-8ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 8. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
uPA Coated Microplate	96 Wells	2-8ºC
10X Wash Buffer	50 mL	2-8ºC
Human PAI1 zero unit activity standard (0 U)	2 Vial	2-8ºC
Human PAI1 high activity standard (260U)	1 Vial	2-8ºC
Assay Diluent	10 mL	2-8ºC
Anti-Human PAI1 Primary Antibody (lyophilized)	1 Vial	2-8ºC
HRP Secondary Antibody	1 Vial	2-8ºC
TMB Substrate Solution	10 mL	2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement.
- Manifold dispenser/aspirator or automated microplate washer.
- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes and Pipette tips.
- Deionized or distilled water.
- Polypropylene tubes for dilution of standard.
- Paper towels or laboratory wipes.
- 1N H_2SO_4 or 1N HCl.
- Bovine Serum Albumin Fraction V (BSA).
- Tris(hydroxymethyl)aminomethane (Tris).
- Sodium Chloride (NaCl).

7. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

8. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

8.1 **1X Tris-Buffered Saline (TBS)**

0.1M Tris, 0.15M NaCl, pH 7.4.

8.2 **1X Blocking Buffer**

3% BSA (w/v) in 1X TBS.

8.3 **1X Wash Buffer**

Dilute 50 mL of 10X Wash Buffer concentrate with 450 mL of deionized water. Mix gently and thoroughly.

8.4 **1X PAI1 Primary Antibody**

Reconstitute Anti-Human PAI1 Primary Antibody to prepare a 1X PAI1 Primary Antibody by adding 11 mL of 1X Blocking Buffer directly to the vial and agitate gently to completely dissolve contents.

8.5 **1X HRP Antibody**

Prepare 1X HRP Antibody by diluting 2 µL of the HRP Secondary Antibody in 10 mL of 1X Blocking Buffer.

Note: The secondary antibody provided in this kit is an HRP conjugated Anti-mouse antibody.

- Reconstituted primary antibody may be stored at -80°C for later use. Do not freeze-thaw the primary antibody more than once.

9. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

9.1 Reconstitute Human PAI1 Negative Standard (0 U) by adding 1 mL of deionized water to each vial and agitate gently to completely dissolve contents. Final concentration is 0 U/mL.

9.2 Reconstitute Human PAI1 Positive Standard (260 U) by adding 1 mL of deionized water to the vial and agitate gently to completely dissolve contents. Final concentration is 260 U/mL.

9.3 Label eleven tubes # 1-11.

9.4 Prepare 100 U/mL **Standard #1,** in tube #1 by adding 30 µL Human PAI1 Positive Standard Stock Solution (260 U/mL) to 48 µL Human PAI1 Negative Standard (0 U/mL) and mix gently and thoroughly.

9.5 Prepare 50 U/mL **Standard #2,** in tube #2 by adding 15 µL Human PAI1 Positive Standard Stock Solution (260 U/mL) to 63 µL Human PAI1 Negative Standard (0 U/mL) and mix gently and thoroughly.

9.6 Prepare 25 U/mL **Standard #3,** in tube #3 by adding 10 µL Human PAI1 Positive Standard Stock Solution (260 U/mL) to 94 µL Human PAI1 Negative Standard (0 U/mL) and mix gently and thoroughly.

9.7 Prepare 10 U/mL **Standard #4,** in tube #4 by adding 3 µL Human PAI1 Positive Standard Stock Solution (260 U/mL) to 75 µL Human PAI1 Negative Standard (0 U/mL) and mix gently and thoroughly.

9.8 Prepare 5 U/mL **Standard #5,** in tube #5 by adding 3 µL Human PAI1 Positive Standard Stock Solution (260 U/mL) to 153 µL Human PAI1 Negative Standard (0 U/mL) and mix gently and thoroughly.

9.9 Prepare 2 U/mL **Standard #6**, in tube #6 by adding 60 µL **Standard #5** (5 U/mL) to 90 µL Human PAI1 Negative Standard (0 U/mL) and mix gently and thoroughly

9.10 Prepare 1 U/mL **Standard #7**, in tube #7 by adding 75 µL **Standard #6** (2 U/mL) to 75 µL Human PAI1 Negative Standard (0 U/mL) and mix gently and thoroughly

9.11 Using the table below as a guide, prepare further serial dilutions for **Standards #8, #9 and #10**.

9.12 Human PAI1 Negative Standard (0 U/mL) serves as the zero standard, 0 ng/mL (Tube #11).

Standard #	Sample to dilute	Volume to dilute (µL)	Human PAI1 Neg Standard (0 U/mL) (µL)	Total Volume (µL)	Starting Conc. (U/mL)	Final Conc. (U/mL)
1	Human PAI1 Positive Standard Stock Solution (260 U/mL)	30	48	78	260	100
2	Human PAI1 Positive Standard Stock Solution (260 U/mL)	15	63	78	260	50
3	Human PAI1 Positive Standard Stock Solution (260 U/mL)	10	94	104	260	25
4	Human PAI1 Positive Standard Stock Solution (260 U/mL)	3	75	78	260	10
5	Human PAI1 Positive Standard Stock Solution (260 U/mL)	3	153	156	260	5
6	Standard #5	60	90	150	5	2
7	Standard #6	75	75	150	2	1
8	Standard #7	75	75	150	1	0.5
9	Standard #8	75	75	150	0.5	0.25
10	Standard #9	75	75	150	0.25	0.125
11	N/A	N/A	75	75	0	0

- Reconstituted standards may be stored at -80°C for later use. Do not freeze-thaw the standard more than once.

10. SAMPLE COLLECTION AND STORAGE

- **Blood** – Collect 9 volumes of blood in 1 volume of 0.1 M trisodium citrate or acidified citrate. Immediately after collection of blood, samples must be centrifuged at 3,000 x g for 15 minutes. It is important to ensure a platelet free preparation as platelets can release PAI1. The plasma must be transferred to a clean plastic tube and must be stored on ice prior to analysis.

  The collected PAI1 activity samples are stable for up to 24 hours or stored at -20ºC for up to one month and thawed three times without loss of PAI1 activity.

  This kit has been validated in Citrate, EDTA, and Heparin collected plasma.

11. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as blanks, omitting primary antibody from well additions.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

12. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards and samples in duplicate.**

12.1. Add 80 µL General Assay Diluent to wells that are going to be used.

12.2. Add 20 µL prepared standards (in duplicate) and samples to wells.

12.3. Shake plate at 300 rpm for 30 minutes.

12.4. Wash wells three times with 300 µL 1X Wash Buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

12.5. Add 100 µL of 1X PAI1 Primary Antibody primary antibody to all wells.

12.6. Shake plate at 300 rpm for 30 minutes.

12.7. Wash wells three times with 300 µL 1X Wash Buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

12.8. Add 100 µL of 1X HRP Antibody to all wells.

12.9. Shake plate at 300 rpm for 30 minutes.

12.10. Wash wells three times with 300 µL 1X Wash Buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

12.11. Add 100 µL TMB Substrate Solution to all wells and shake plate for 2-8 minutes. Substrate will change from colorless to different strengths of blue.

12.12. Quench reaction by adding 50 µL of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

12.13. Set the absorbance at 450 nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450 nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

13. CALCULATIONS

Plot A_{450} against the amount of PAI1 in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of PAI1 in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

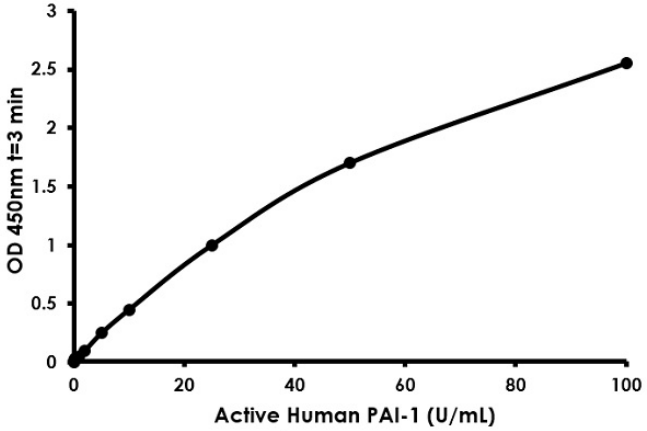


Figure 1. ab157528 PAI1 Human ELISA kit Active Human PAI1 Full Range

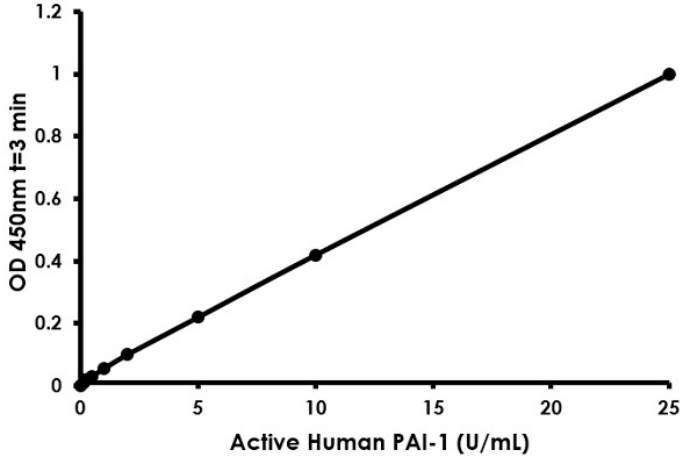


Figure 2. ab157528 PAI1 Human ELISA kit Active Human PAI1 Linear Range

15. TYPICAL SAMPLE VALUES

SENSITIVITY -

The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD_{450}: 0.057-0.067) and calculating the corresponding concentration. The MDD was 0.11 U/mL.

RECOVERY –

(Sample spiking at a range of concentrations in representative sample matrices)

Sample Type	Average % Recovery	Range
1	100	96.9 - 105%
2	101	95 - 106%
3	96.7	92.8 - 104%
4	88.1	83.5 - 91.5%

LINEARITY OF DILUTION -

Plasma Dilution	% Expected Value
1:2	90.8
1:4	83.9
1:8	98.2
1:16	98

PRECISION –

	Intra-Assay	Inter-Assay
n =	20	10
Mean Sample Conc. (U/mL)	2.80	4.20
SD	0.257	0.399
%CV	9.18	9.52

16. ASSAY SPECIFICITY

This assay recognizes natural active Human PAI1. Pooled normal plasma from Sheep was assayed, and no significant cross-reactivity was observed. Pooled normal plasma from Mouse resulted in significant color development.

17. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

18. NOTES

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)