

# **ab198509 – PAI1 (SERPINE1) Rat ELISA Kit**

Instructions for Use

For the quantitative measurement of active PAI1 (SERPINE1) in rat plasma and serum.

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

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## 1. BACKGROUND

Abcam's PAI1 (SERPINE1) *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of active PAI1 (SERPINE1) in rat plasma and serum.

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 and will not be detected. Unbound PAI1 samples are washed away and an anti-PAI1 primary antibody is added. Excess primary antibody is washed away and bound antibody, which is proportional to the original active PAI1 present in the samples, is then reacted with the horseradish peroxidase secondary antibody. Following an additional washing step, TMB is then used for color development at 450 nm. The amount of color development is directly proportional to the concentration of active PAI1 in the sample.

Plasminogen activator inhibitor-1 (PAI1) is a serine protease inhibitor that is an important regulator of fibrinolysis and extracellular matrix turnover. PAI1 may be important in hepatocyte growth and proliferation *in vivo*. Increased PAI1 levels may increase the risk for myocardial infarction, atherosclerosis, and retinosis. Increased PAI1 levels may also play an important role in the development and pathogenesis of diabetic nephropathy. Decreased levels may reduce thrombotic events.

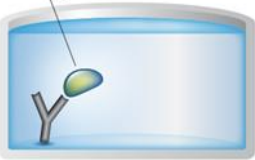
## 2. ASSAY SUMMARY

### Primary capture antibody



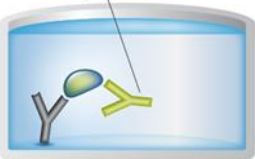
Prepare all reagents, samples, and controls as instructed. Plate is supplied pre-coated with capture antibody.

### Sample



Add sample to appropriate wells. Incubate at room temperature.

### Primary detector antibody



Add primary detection antibody. Incubate at room temperature.

### HRP conjugated antibody



Aspirate and wash each well. Add HRP conjugated secondary antibody, which binds the primary antibody. Incubate at room temperature.

### Substrate **Colored product**



Aspirate and wash each well. Add developing solution until color develops and then add the stop solution. Immediately begin recording the color development.

## 3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

## 4. STORAGE AND STABILITY

Store all kit components at +2-8°C upon arrival.

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

## 5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
uPA Antibody pre-coated 96 well microplate (12 x 8 well strips)	1X 96 tests	+2-8°C
10X Wash Buffer	1X 50 mL	+2-8°C
Rat PAI1 activity standard	1 Vial	+2-8°C
Anti-rat PAI1 primary antibody	1 Vial	+2-8°C
Anti-rabbit horseradish peroxidase secondary antibody	1 Vial	+2-8°C
TMB substrate solution	1X 10 mL	+2-8°C

### **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<sub>2</sub>SO<sub>4</sub> or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

### **7. LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

### 8. 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.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- 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, control 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.**

## 9. REAGENT PREPARATION

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

### 9.1. **1X Wash Buffer**

Dilute 50 mL of 10X Wash Buffer with 450 mL of deionized water.

### 9.2. **1X TBS Buffer**

0.1M Tris, 0.15M NaCl, pH 7.4

### 9.3. **1X Blocking Buffer**

3% BSA (w/v) in TBS.

### 9.4. **PAI Primary Antibody**

Reconstitute PAI Primary Antibody by adding 10 mL of 1X Blocking Buffer directly to the vial and agitate gently to completely dissolve contents. Reconstituted PAI Primary Antibody may be stored at -80°C for later use.



## 10. STANDARD PREPARATIONS

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

- 10.1 Reconstitute PAI Standard by adding 5 mL of 1X Blocking Buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 50 ng/mL **Standard #1**.
- 10.2 Label nine further tubes # 2-10.
- 10.3 Prepare **Standard #2**, in tube #2 by adding 500  $\mu$ L (50 ng/mL) **Standard #1** to 500  $\mu$ L 1X Blocking Buffer and mix gently and thoroughly.
- 10.4 Using the table below as a guide, prepare further serial dilutions using 1X Blocking Buffer as diluent.
- 10.5 1X Blocking Buffer serves as the zero standard, 0 ng/mL (tube #10).

## Standard Dilution Preparation Table

Standard #	Sample to Dilute	Volume to Dilute (μL)	Volume of Diluent (μL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	See section 10.1				50
2	Standard #1	500	500	50	25
3	Standard #2	400	600	25	10
4	Standard #3	500	500	10	5
5	Standard #4	400	600	5	2
6	Standard #5	500	500	2	1
7	Standard #6	500	500	1	0.5
8	Standard #7	500	500	0.5	0.25
9	Standard #8	400	600	0.25	0.1
10 (Blank)	-	-	500	-	0



## 11. SAMPLE COLLECTION AND STORAGE

- 11.1 Collect 9 volumes of blood in 1 volume of a 3.8% trisodium citrate or acidified citrate.
- 11.2 Immediately after collection of blood, samples must be centrifuged at 3000 x g for 15 minutes. It is important to ensure a platelet free preparation since platelets can release PAI1.
- 11.3 The plasma must be stored on ice prior to analysis. The PAI1 activity samples collected are stable for up to 24 hours or stored at -20°C for up to one month and thawed three times without loss of PAI1 activity.

## 12. 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 +2-8°C.
- For each assay performed, a minimum of 2 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.

## **13. ASSAY PROCEDURE**

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
- **Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at +2-8°C.**

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove microtiter plate from bag and add 100  $\mu$ L PAI1 standards (in duplicate) and samples to wells.

13.3 Carefully record position of standards and samples.

13.4 Shake plate at 300 rpm for 30 minutes.

13.5 Wash wells three times with 300  $\mu$ L 1X Wash Buffer. Remove excess 1X Wash Buffer by gently tapping plate on paper towel.

*NOTE: The assay measures active PAI1 in the 0.1-50 ng/mL range. Plasma and serum samples should be applied directly to the plate without dilution.*

13.6 Add 100  $\mu$ L PAI Primary Antibody to all wells.

13.7 Shake plate at 300 rpm for 30 minutes.

13.8 Wash wells three times with 300  $\mu$ L 1X Wash Buffer. Remove excess 1X Wash Buffer by gently tapping plate on paper towel.

13.9 Dilute 1  $\mu$ L of HRP-conjugated Secondary Antibody in 10 mL of Blocking Buffer and add 100  $\mu$ L to all wells.

13.10 Shake plate at 300 rpm for 30 minutes.

## ASSAY PROCEDURE

- 13.11 Wash wells three times with 300  $\mu\text{L}$  1X Wash Buffer. Remove excess 1X Wash Buffer by gently tapping plate on paper towel.
- 13.12 Add 100  $\mu\text{L}$  TMB substrate to all wells and shake plate for 2-5 minutes. Substrate will change from colorless to different strengths of blue.
- 13.13 Quench reaction by adding 50  $\mu\text{L}$  of 1N  $\text{H}_2\text{SO}_4$  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.
- 13.14 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 ( $A_{450}$ ).

## 14. 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 Samples can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

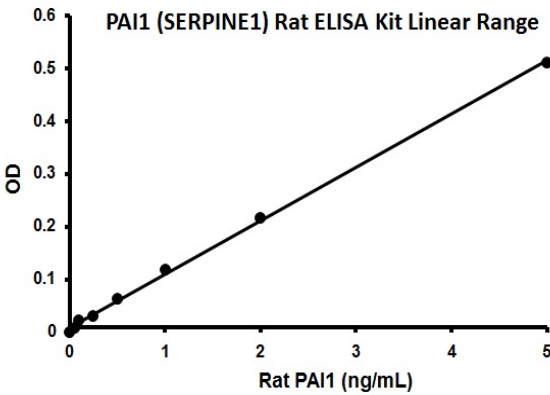
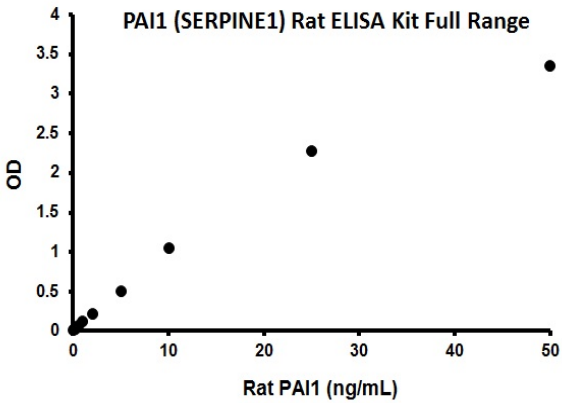
The concentration level of PAI1 in rat plasma was found to be  $1.0 \pm 0.5$  ng/mL for active PAI1 and  $1.8 \pm 0.9$  ng/mL for PAI1 antigen.

Abnormalities in PAI1 levels have been reported in the following conditions:

- Artherosclerosis: Increased PAI1 levels may contribute to artherosclerosis.
- Diabetes: Elevated PAI1 levels in rats may contribute to the development and pathogenesis of diabetic nephropathy.
- Myocardial Infarction: Increased PAI1 levels may contribute to myocardial infarction.
- Restenosis: Increased PAI1 levels is associated with restenosis.
- Thrombosis: Decreased PAI1 levels may reduce thrombotic events.
- Deep Venous Thrombosis: Elevated PAI1 levels may be associated with deep venous thrombosis.
- Coronary Artery Disease: Elevated PAI1 levels may increase the risk of coronary artery disease.
- Endotoxemia: Endotoxin induces a large increase in PAI1 levels (100- to 200-fold).

## 15. TYPICAL DATA

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



## 16. TYPICAL SAMPLE VALUES

### INTRA-ASSAY PRECISION

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/mL)	0.957	2.25	15.6
Standard Deviation	0.088	0.134	1.01
CV (%)	9.24	5.97	6.48

### INTER-ASSAY PRECISION

Three samples of known concentration were tested in ten independent assays to assess inter-assay precision.

Sample	1	2	3
n	10	10	10
Mean (ng/mL)	0.926	1.40	9.17
Standard Deviation	0.113	0.096	0.858
CV (%)	12.2	6.86	9.36

### 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<sub>450</sub>: 0.124-0.136) and calculating the corresponding concentration. The MDD was 0.079 ng/mL.



## RECOVERY

The recovery of antigen spiked to levels throughout the range of the assay in PAI1 depleted plasma was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/mL)	0.721	2.24	13.3	21.5
Average % Recovery	103	102	102	108
Range	97 - 109%	96 - 105%	98 - 105%	97 - 112%

## LINEARITY OF DILUTION

To assess the linearity of the assay, pooled citrated rat plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay. Plasma samples did not demonstrate linearity and should be analyzed without dilution.

## 17. ASSAY SPECIFICITY

This assay recognizes natural and recombinant active rat PAI1. Pooled normal plasma from mouse and dog was assayed and significant cross-reactivity was observed. Pooled normal plasma from human and pig was assayed and minor cross-reactivity was observed. Pooled normal plasma from sheep was assayed and no significant cross-reactivity was observed. Pooled normal plasma from rabbit resulted in significant color development.

## 18. TROUBLESHOOTING

<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
Poor standard curve	Inaccurate pipetting Improper standards dilution	Check pipettes 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 2-8°C. Keep substrate solution protected from light.

19. NOTES

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