ab197752 – PAI1 (SERPINE1) Mouse SimpleStep ELISA® Kit

Instructions for Use

For the quantitative measurement of PAI1 (SERPINE1) in mouse serum, plasma, and cell culture supernatants.

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

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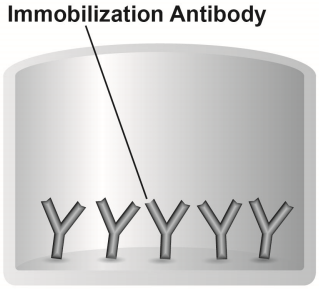
1. BACKGROUND

PAI1 (SERPINE1) *in vitro* SimpleStep ELISA®™ (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of PAI1 protein in mouse serum, plasma and cell culture supernatant.

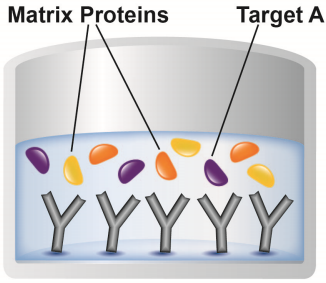
The SimpleStep ELISA®™ employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Plasminogen activator inhibitor-1 (PAI1 or Serpin E1) is encoded by the Serpine1 gene. PAI1 is a serine protease inhibitor with a major role in the regulation of fibrinolysis. PAI1 is the principal inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA), both of which are activators of plasminogen. PAI1 is a secreted protein produced by the endothelium as well as adipose tissue.

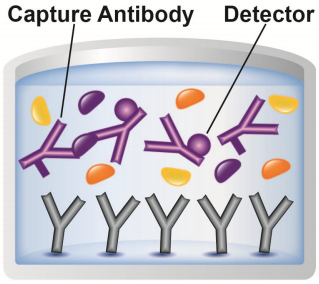
2. ASSAY SUMMARY



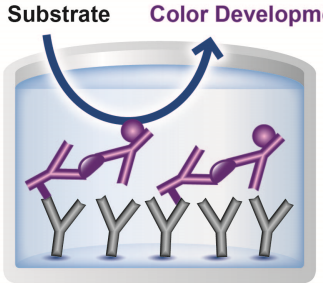
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.



Add standard or sample to appropriate wells.



Add Antibody Cocktail to all wells. Incubate at room temperature.



Aspirate and wash each well. Add TMB Substrate to each well and incubate. Add Stop Solution at a defined endpoint. Alternatively, record color development kinetically after TMB substrate addition.

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 I n loss of performance.

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 the Reagent and Standard Preparation sections.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
10X Mouse PAI1 Capture Antibody	1 x 600 µL	+2-8ºC
10X Mouse PAI1 Detector Antibody	1 x 600 µL	+2-8ºC
PAI1 Mouse Lyophilized Recombinant Protein	2 Vials	+2-8ºC
Antibody Diluent CPI	1 x 6 mL	+2-8ºC
10X Wash Buffer PT	20 mL	+2-8ºC
TMB Substrate	12 mL	+2-8ºC
Stop Solution	12 mL	+2-8ºC
Sample Diluent NS	50 mL	+2-8ºC
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+2-8ºC
Plate Seal	1	+2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Microplate reader capable of measuring absorbance at 450 or 600 nm
- Method for determining protein concentration (BCA assay recommended)
- Deionized water
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4)
- Multi- and single-channel pipettes
- Tubes for standard dilution
- Plate shaker for all incubation steps
- Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors)

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
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11)
- All samples should be mixed thoroughly and gently
- Avoid multiple freeze/thaw of samples
- Incubate ELISA plates on a plate shaker during all incubation steps
- When generating positive control samples, it is advisable to change pipette tips after each step
- The provided Antibody Diluents and Sample Diluents contain protease inhibitor aprotinin. Additional protease inhibitors can be added if required
- **To avoid high background always add samples or standards to the well before the addition of the antibody cocktail**
- **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 to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. **The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.**
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

9.1 1X Wash Buffer PT

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

9.2 Antibody Cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CPI. Mix thoroughly and gently.

10. STANDARD PREPARATION

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

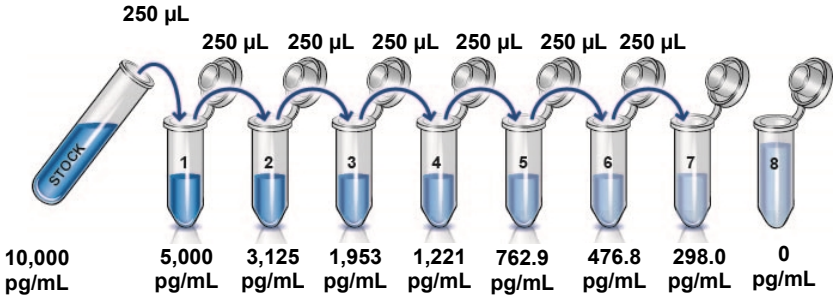
The following table describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Reconstitute the PAI1 mouse lyophilized recombinant standard sample by adding 1 mL Sample Diluent NS by pipette. Mix thoroughly and gently. Hold at room temperature for 10 minutes and mix gently. This is the 10,000 pg/mL **Stock Standard** Solution.

10.2 Label eight tubes, Standards 1– 8.

10.3 Add 250 µL Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



11. SAMPLE PREPARATION

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range**
Mouse Serum	0.2-25%
Mouse Plasma - Citrate	0.2-25%
L929 Cell Culture Supernatant	1-10%

11.1 **Plasma**

Collect plasma and centrifuge samples at 2,000 x g for 10 minutes. Dilute samples into Sample Diluent NS and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 **Serum**

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples into Sample Diluent NS and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

11.3 **Cell Culture Supernatants**

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

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 plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C
- For each assay performed, a minimum of two wells must be used as the zero control
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Differences in well absorbance or “edge effects” have not been observed with this assay

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.**

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

13.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

13.3 Add 50 µL of all sample or standard to appropriate wells.

13.4 Add 50 µL of the Antibody Cocktail to each well.

13.5 Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6 Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7 Add 100 µL of TMB Substrate to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

13.8 Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nm
Time:	up to 15 min
Interval:	20 sec - 1 min
Shaking:	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

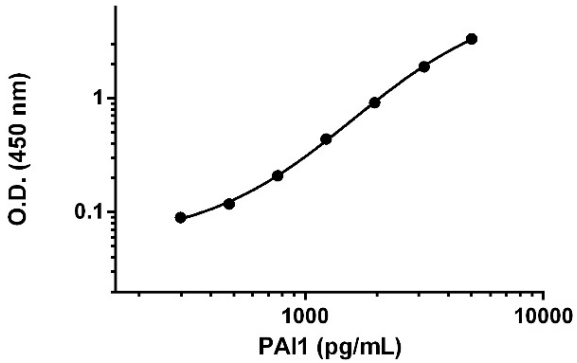
13.9 Analyze the data as described below.

14. CALCULATIONS

Subtract average zero standard from all readings. Average the duplicate readings of the positive control dilutions and plot against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

15. TYPICAL DATA

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



Standard Curve Measurements			
Conc. (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.098	0.096	0.096
298.0	0.150	0.150	0.150
476.8	0.181	0.176	0.179
762.9	0.272	0.267	0.270
1,221	0.508	0.492	0.500
1,953	0.997	0.954	0.975
3,125	1.942	1.950	1.946
5,000	3.396	3.367	3.381

Figure 1. Example of PAI1 standard curve. The PAI1 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The calculated minimal detectable dose (MDD) is 200 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=17) and adding 2 standard deviations then extrapolating the corresponding concentrations.

RECOVERY –

Three concentrations of PAI1 were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
10% Mouse Serum	109	90-121
10% Mouse Plasma - Citrate	104	96-111
1%Cell Culture Media (with serum)	83	72-88
1%Cell Culture Media (serum free)	117	104-128

LINEARITY OF DILUTION –

Native PAI1 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Dilution Factor	Interpolated value	25% Mouse Serum	25% Mouse Plasma (Citrate)	10% L929 Supernatant
Undiluted	pg/mL	4,818	1,902	3,115
	% Expected value	**100**	**100**	**100**
2	pg/mL	2,315	983	1,521
	% Expected value	**95**	**103**	**98**
4	pg/mL	1,137	535	765
	% Expected value	**94**	**112**	**98**
8	pg/mL	606.7	255	415
	% Expected value	**101**	**107**	**107**
16	pg/mL	326.4	98	205
	% Expected value	**108**	**82**	**105**

PRECISION –

Mean coefficient of variations of interpolated values from 3 concentrations of mouse serum within the working range of the assay.

	Intra-Assay	Inter-Assay
n=	5	3
CV (%)	6.7	14.2

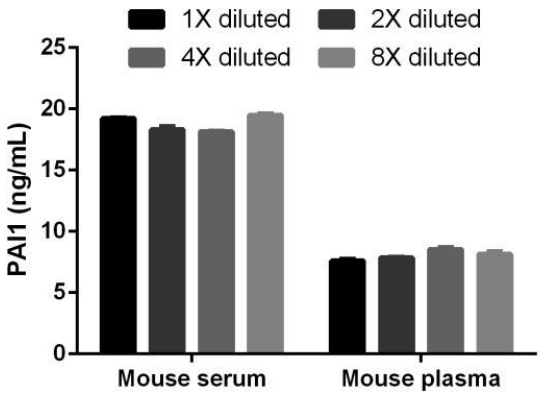


Figure 2. Interpolated concentrations of PAI1 in mouse serum and plasma (citrate). The concentrations of PAI1 were measured in duplicate and interpolated from the PAI1 standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean PAI1 concentration was determined to be 18.98 ng/mL in mouse serum and 7.69 ng/mL in mouse plasma (citrate).

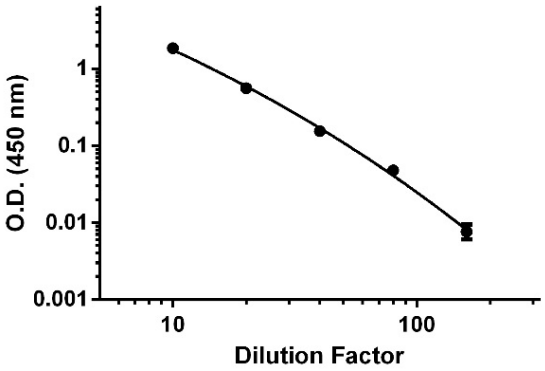


Figure 3. Demonstration of the linearity of dilution of the assay by titrating of L929 Stimulated for 3-days with PMA/PHA diluted 10-fold to 80-fold diluted in Sample Diluent NS. Background subtracted data values (mean +/- SD, n = 2) are graphed.

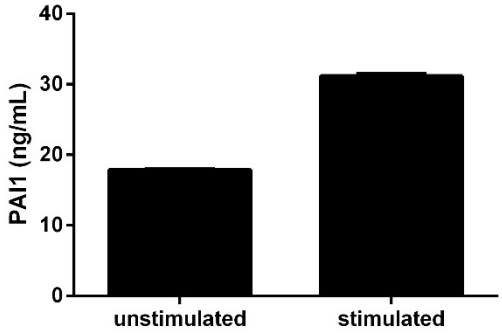


Figure 4. Comparison of secreted PAI1 in unstimulated and PMA/PHA-stimulated L929 Cells. L929 cells were grown in the absence (unstimulated) or presence of Phorbol Myristate Acetate (PMA) and phytohemagglutinin (PHA) (stimulated) for 3 days. PAI1 was measured in 10-fold diluted cell culture supernatants of unstimulated and PMA/PHA stimulated L929 and cell culture media. Measured values were interpolated from the PAI1 Standard Curve diluted in Sample Diluent NS and corrected for dilution factor. Mean of duplicate values +/-SD are graphed: 17.9 ng/mL unstimulated, 31.1 ng/mL stimulated, and undetectable in media.

17. ASSAY SPECIFICITY

This kit recognizes both native and recombinant Mouse mouse PAI1 protein in serum, plasma and cell culture supernatant.

Cell and tissue extract samples have not been tested with this kit.

18. SPECIES REACTIVITY

This kit recognizes mouse PAI1 (SERPINE1) protein.

CROSS REACTIVITY

The recombinant Human human PAI1 was prepared at 5 ng/mL in Sample Diluent NS and assayed for cross reactivity. 100% cross-reactivity was observed.

Other species reactivity was determined by measuring 10 fold diluted serum samples of various species, interpolating the protein concentrations from the mouse standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in mouse serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:

Rabbit

Chicken

Please contact our Technical Support team for more information

19. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate Pipetting	Check pipettes
	Improper standard 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; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of Stop solution
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 your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.
Precipitate in Diluent	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37ºC.

20. **NOTES**

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