



Sino Biological  
Biological Solution Specialist

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# Human SerpinE1 / PAI-1 ELISA Pair Set

**Catalog Number : SEK10296**

To achieve the best assay results, this manual must be read carefully before using this product and the assay is run as summarized in the General ELISA protocol.

## BACKGROUND

Plasminogen activator inhibitor 1, also known as PAI-1, Endothelial plasminogen activator inhibitor, SerpinE1 and PLANH1, is a secreted glycoprotein which belongs to the serpin family. SerpinE1 is the primary physiological inhibitor of the two plasminogen activators urokinase (uPA) and tissue plasminogen activator (tPA). Its rapid interaction with TPA may function as a major control point in the regulation of fibrinolysis. Defects in SerpinE1 are the cause of plasminogen activator inhibitor-1 deficiency (PAI-1 deficiency) which is characterized by abnormal bleeding due to SerpinE1 defect in the plasma. High concentrations of SerpinE1 have been associated with thrombophilia which is an autosomal dominant disorder in which affected individuals are prone to develop serious spontaneous thrombosis. Studies of PAI-1 have contributed significantly to the elucidation of the protease inhibitory mechanism of serpins, which is based on a metastable native state becoming stabilised by insertion of the RCL into the central beta-sheet A and formation of covalent complexes with target proteases. Greater expression of PAI-1 has been associated with increased survival of cells and resistance to apoptosis. PAI-1 appears to influence apoptosis by decreasing cell adhesion (anoikis) as well as its effect on intracellular signaling. PAI-1, in its active state, also binds to the extracellular protein vitronectin. When in complex with its target proteases, it binds with high affinity to endocytosis receptors of the low density receptor family. The mechanisms of PAI-1 overexpression during obesity are complex, and it is conceivable that several inducers are involved at the same time at several sites of synthesis. PAI-1 is also implicated in adipose tissue development. It suggests that PAI-1 inhibitors serve in the control of atherothrombosis.

## PRINCIPLE OF THE TEST

The Sino Biological ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for Human SerpinE1 / PAI-1 coated on a 96-well plate. Standards and samples are added to the wells, and any Human SerpinE1 / PAI-1 present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated rabbit anti-Human SerpinE1 / PAI-1 monoclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". The wells are again washed and TMB substrate solution is loaded, which produces color in proportion to the amount of Human SerpinE1 / PAI-1 present in the sample. To end the enzyme reaction, the stop solution is added and absorbances of the microwell are read at 450 nm.

## INTENDED USE

- ◆The Human SerpinE1 / PAI-1 ELISA Pair Set is for the quantitative determination of Human SerpinE1 / PAI-1.
- ◆This ELISA Pair Set contains the basic components required for the development of sandwich ELISAs.

## ASSAY PROCEDURE SUMMARY



**This Pair Set has been configured for research use only and is not to be used in diagnostic procedures.**

## MATERIALS PROVIDED

**Bring all reagents to room temperature before use.**

**Capture Antibody** – 1 mg/mL of mouse anti-Human SerpinE1 / PAI-1 monoclonal antibody (in PBS, pH 7.4). Dilute to a working concentration of 1 µg/mL in PBS before coating. (Catalog: # 10296-MM01)

**Detection Antibody** – 0.2 mg/mL of rabbit anti-Human SerpinE1 / PAI-1 monoclonal antibody conjugated to horseradish-peroxidase (HRP) (in PBS, 50 % HRP-Protector, pH 7.4, store at 4°C). Dilute to working concentration of 0.03125 µg/mL in detection antibody dilution buffer before use. (Catalog: # 10296-R004)

**Standard** – Each vial contains 125 ng of recombinant Human SerpinE1 / PAI-1. Reconstitute with 1 mL detection antibody dilution buffer. After reconstitution, store at -20°C to -80°C in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 3000 pg/mL is recommended.

## SOLUTIONS REQUIRED

**PBS** - 136.9 mM NaCl, 10.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 2.7 mM KCl, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4, 0.2 µm filtered

**TBS** - 20 mM Tris, 150 mM NaCl, pH 7.4

**Wash Buffer** - 0.05% Tween20 in TBS, pH 7.2 - 7.4

**Blocking Buffer** - 2% BSA in Wash Buffer

**Sample dilution buffer** - 0.1% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

**Detection antibody dilution buffer** - 0.5% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

**Substrate Solution** : To achieve best assay results, fresh substrate solution is recommended

**Substrate stock solution** - 10mg / ml TMB ( Tetramethylbenzidine ) in DMSO

**Substrate dilution buffer** - 0.05M Na<sub>2</sub>HPO<sub>4</sub> and 0.025M citric acid ; adjust pH to 5.5

**Substrate working solution** - For each plate dilute 250 µl substrate stock solution in 25ml substrate dilution buffer and then add 80 µl 0.75% H<sub>2</sub>O<sub>2</sub> , mix it well

**Stop Solution** - 2 N H<sub>2</sub>SO<sub>4</sub>

## PRECAUTION

The Stop Solution suggested for use with this Pair Set is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

## STORAGE

**Capture Antibody:** Aliquot and store at  $-20^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$  for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

**Detection Antibody:** Store at  $4^{\circ}\text{C}$  and protect it from prolonged exposure to light for up to 6 months from date of receipt. **DO NOT FREEZE!**

**Standard:** Store lyophilized standard at  $-20^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$  for up to 6 months from date of receipt. Aliquot and store the reconstituted standard at  $-80^{\circ}\text{C}$  for up to 1 month. Avoid repeated freeze-thaw cycles.

## GENERAL ELISA PROTOCOL

### Plate Preparation

1. Dilute the capture antibody to the working concentration in PBS. Immediately coat a 96-well microplate with 100µL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4°C.
2. Aspirate each well and wash with at least 300µl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

### Assay Procedure

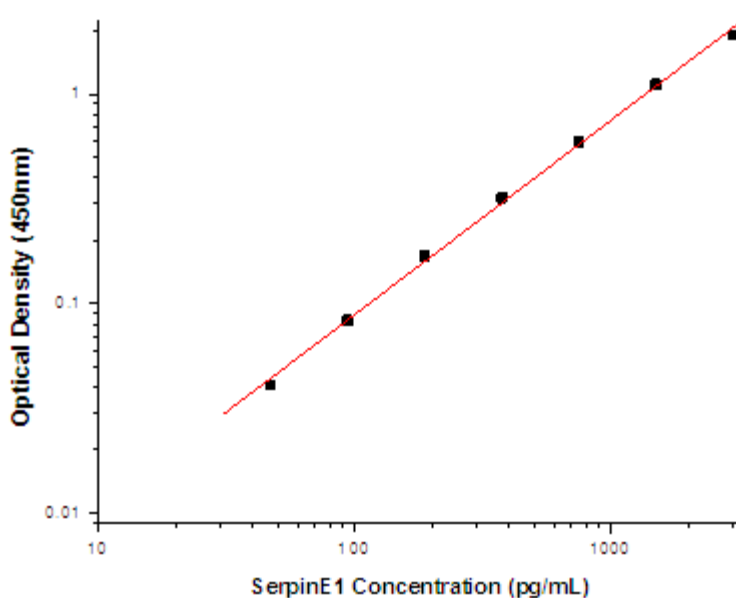
1. Add 100 µL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 µL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 200 µL of substrate solution to each well. Incubate for 20 minutes at room temperature ( **if substrate solution is not as requested, the incubation time should be optimized** ). Avoid placing the plate in direct light.
6. Add 50 µL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

## CALCULATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
- Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
- To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

## TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.



Concentration ( pg/mL)	Zero standard subtracted OD
0	0
46.88	0.041
93.75	0.083
187.50	0.168
375	0.321
750	0.589
1500	1.114
3000	1.926

## PERFORMANCE CHARACTERISTIC

### SENSITIVITY

The minimum detectable dose of Human SerpinE1 / PAI-1 was determined to be approximately **46.88 pg/ml**. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

# TROUBLE SHOOTING

Problems	Possible Sources	Solutions
<b>No signal</b>	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
<b>Poor Standard Curve</b>	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 °C
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
<b>Poor detection value</b>	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
<b>High Background</b>	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated.	Use clean plates, tubes and pipettes tips
<b>Non-specificity</b>	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples



## ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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**Notes**