

INSTRUCTIONS

Directions for ELISA assay kit IHPAIKT-TOT™ for the detection of total Human PAI-1

Reagents and supplies needed for assay:

- 1) ELISA microtiter plate (Immulon 2 - 96 wells) - (user coats with 100 μ l of a 5 μ g/ml capture antibody provided)
- 2) Capture antibody (provided): Dilute into 10ml TBS buffer according to vial instructions to achieve a 5 μ g/ml solution.
- 3) TBS (not provided):
0.10 M TRIS
0.15 M sodium chloride
pH 7.4
- 4) Wash buffer: TBS/Tween: 0.05 % Tween 20 in TBS; 0.1% BSA (not provided)
- 5) Blocking buffer : 3.0% BSA (bovine serum albumin) in TBS (not provided):
- 6) Human PAI-1 Standard (for standard curve - provided)
- 7) 2° antibody (provided): Anti-human PAI-1 IgG biotinylated.
Working solution: dilute the 20 μ l vial to 10 ml in blocking buffer **OR ENOUGH AS REQUIRED**).
- 8) Avidin HRP reagent: avidin horseradish peroxidase
Working solution: 1 vial diluted according to instructions in blocking buffer
- 9) TMB substrate Solution: 10 ml provided

Principal of the assay method:

Human PAI-1 binds to the capture antibody coated on the micro titer plate. Free, latent and complexed human PAI-1 will bind to the capture antibody. After appropriate washing steps, biotinylated anti-human PAI-1 secondary antibody binds to the captured PAI-1. Excess antibody is washed away, and bound antibody is then reacted with the avidin horseradish peroxidase reagent. TMB substrate is used for color development at 450 nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of purified human PAI-1. Suggested dilutions of human PAI-1 for standard curve are:

0; 0.05; 0.1; 0.25; 0.5, 1.0; 3.0; 5.0; 10.0; 25.0; 50.0 ng/ml

Protocol:

NOTE: Vigorously shake the ELISA plate at each incubation step.

1) Remove the desired quantity of capture antibody from the vial provided and coat wells with 100 μ l of 5 μ g/ml antibody in TBS buffer. Coat the wells overnight at 4° C or at room temperature for at least 4 hours.

2) Aspirate off excess capture antibody solution. Wash the wells three times with 0.3ml wash buffer, and then blocked with the addition of 0.3ml of blocking buffer followed by incubation for 1 hour at room temperature (alternatively, Pierce brand Superblock™ may be used to shorten this time).

3) The blocking buffer is aspirated and 100 μ l of each of the standard (make dilutions in blocking buffer) and unknown samples are added to the wells and allowed to incubate for 30 minutes.

4) Aspirate and wash three times with 0.3ml of wash buffer.

5) 100 μ l of the secondary antibody (working solution) is added to each of the wells, and allowed to incubate for 30 min. at 25°C. Dilutions are made in blocking buffer.

6) Aspirate and wash three times with 0.3ml of wash buffer.

7) 100 μ l of the Avidin HRP reagent (working solution) is added to each of the wells and allowed to incubate for 30 minutes at 25°C. Two dilutions must be made before using:

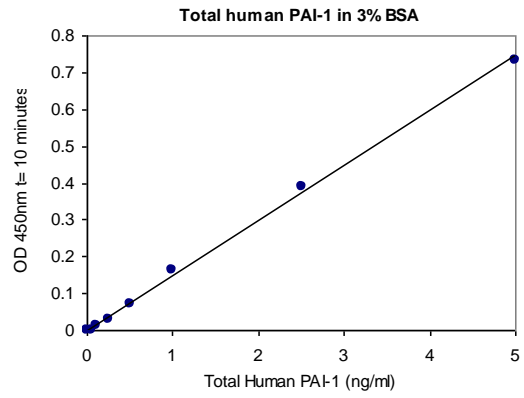
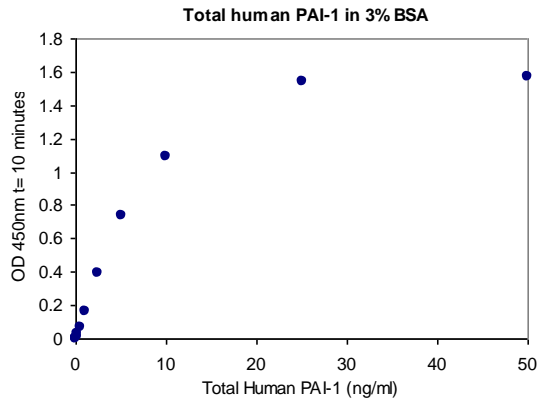
495µl (blocking buffer) + 5µl (avidin HRP)
10ml (blocking buffer) + 5µl (of above)

8) Aspirate and wash three times with 0.3ml of wash buffer.

9) Add 100µl of TMB substrate working solution to each of the wells. The rates of absorbance change at 655nm are measured in the first five minutes. Alternatively, the reaction may be quenched 5-10 minutes by the addition of 50µl of 1N H₂SO₄ to each of the wells, and final absorbance values read at 450nm.

Stock solutions (not the final dilutions) of the capture, human PAI-1, secondary antibody and avidin HRP reagent must be kept frozen (-20°C or below) before use. The TMB substrate solution must be kept refrigerated at 4°C. The PAI-1 standard stocks should be immediately refrozen after use.

A Typical standard curve is shown:



Trouble shooting tips and suggestions:

- 1) Keep capture antibody, Human PAI-1 standard, secondary antibody, and avidin HRP reagent frozen at -70C until ready for use. Keep TMB substrate solution refrigerated at 4°C until ready for use.
- 2) Make the dilutions of PAI-1 standard in the blocking buffer (serial dilution's required -- check calculations twice). These dilutions are quite extensive.
- 3) Be certain you are using the correct antibody at the appropriate place in the protocol. The first antibody is a polyclonal that captures the human PAI-1. The second antibody is a biotinylated monoclonal specific for the human PAI-1.
- 4) If you will not be using the entire ELISA plate, then use only portions of the capture and secondary antibodies, and keep the rest as concentrated stock solutions rather than dilutions.
- 5) If using end point detection, then stop the reaction when the color development is suitable for measurement (visual inspection).
- 6) Take care not to contaminate the TMB substrate solution; if the solution is blue before use, DO NOT USE IT.

REAGENTS FOR HPAIKT-TOT – TOTAL HUMAN PAI-1 ELISA KIT Lot HPAIKT-TOT-603

ELISA Plate	--	1 Immulon 2 (strip well format)
Capture Antibody	--	Lot CA-903 Dilute 29ul into 10 ml TBS and coat with 100 μ I/well
Human PAI-1 (standard)	--	Lot PAI-L-603A
MW	--	43,000
active concentration	--	2.5 mg/ml
volume/aliquot	--	1 X 0.035 ml
buffer	--	0.05M Sodium Phosphate; 0.15M NaCl; 1mM EDTA; pH 6.6
Secondary antibody	--	Anti-human PAI-1 monoclonal IgG (biotinylated)
Lot#	--	MAH-602
volume/aliquot	--	Dilute 20 μ l into 10ml for assay
Avidin HRP reagent	--	Avidin horseradish peroxidase
Lot#	--	AHRP-603
Volume/aliquot	--	1 X 0.01ml
Substrate	--	10 ml
Lot	--	20909