invitrogen

Product Information Sheet

Human PAI-1 ELISA Kit

Catalog nos. KHC3071
KHC3072

Pub. No. MAN0014692 **Rev** 1.0

Description

The Human PAI-1 ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of Hu PAI-1 in serum, EDTA and heparin plasma, buffered solution, and tissue culture medium. The assay will recognize both natural and recombinant Hu PAI-1.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2°C to 8°C.

Components	Cat. no. KHC3071 96 tests	Cat. no. KHC3072 192 tests
Hu PAI-1 Antibody Coated Wells. 96 well plate.	1 plate	2 plates
Hu PAI-1 Biotin Conjugate (biotin-labeled anti-PAI-1). Contains 0.1% sodium azide.	11 mL	2 × 11 mL
Hu PAI-1 Standard. Lyophilized, recombinant Hu PAI-1. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials	4 vials
Wash Buffer Concentrate (25X).	100 mL	100 mL
Standard Diluent Buffer. Contains 0.1% sodium azide.	25 mL	2 × 25 mL
Streptavidin-HRP (100X). Contains 3.3 mM thymol.	0.125 mL	2 × 0.125 mL
Streptavidin-HRP Diluent. Contains 3.3 mM thymol.	25 mL	25 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB).	25 mL	25 mL
Stop Solution.	25 mL	25 mL
Adhesive Plate Covers.	4	8

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Materials required but not provided

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

General Guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com/techresources** for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Manufacturing Site • 7335 Executive Way • Frederick • MD 21704 • E-mail: **techsupport@lifetech.com**

Prepare 1X Wash Buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
3. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

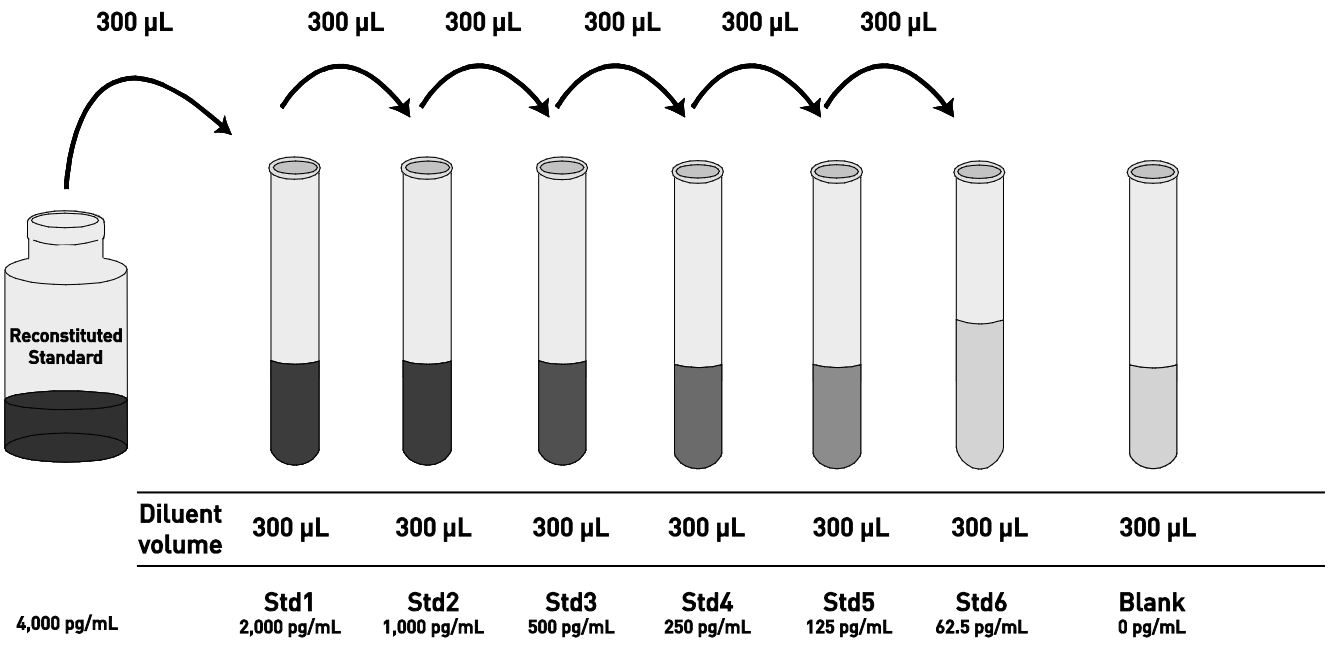
Dilute samples

- Dilute **serum**, **plasma**, and **cell culture medium** two-fold in Standard Diluent Buffer. For these samples, **either** pre-dilute by adding 120 µL of sample, followed by 120 µL of Standard Diluent Buffer in a clean microfuge tube **or** dilute in the well as directed in the ELISA procedure on page 3.
- Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Hu PAI-1 Standard to 4,000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 4,000 pg/mL Hu PAI-1. **Use the standard within 15 minutes of reconstitution**.
2. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 2,000; 1,000; 500; 250; 125; 62.5; and 0 pg/mL Hu PAI-1.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

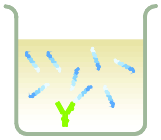
1. Allow Streptavidin-HRP (100X) to reach room temperature and mix gently.
2. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean, absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
3. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 5 hours.**

IMPORTANT! Perform a standard curve with each assay.

Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2 to 8°C for future use.

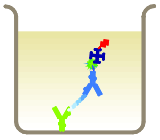


Bind antigen

1. Add 100 µL of Standard Diluent Buffer to zero wells except the chromogen blanks.
2. Add 100 µL of standards, pre-diluted samples (see **Dilute samples**) or controls to the appropriate wells. Alternatively, samples may be diluted directly in the microtiter well by adding 50 µL of Standard Diluent Buffer to each well followed by 50 µL of sample.
3. Cover the plate with the plate cover and incubate for 2 hours at room temperature.
4. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

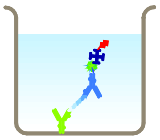
Add Biotin Conjugate

5. Add 100 µL Hu PAI-1 Biotin Conjugate solution into each well except the chromogen blanks.
6. Tap the side of the plate to mix. Cover the plate with plate cover and incubate for 2 hours at room temperature.
7. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.



Add Streptavidin-HRP

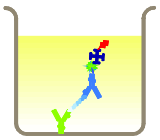
8. Add 100 µL Streptavidin-HRP (see page 2) into each well except the chromogen blanks.
9. Cover the plate with the plate cover and incubate for 30 minutes at room temperature.
10. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.



Add chromogen

11. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
12. Incubate for 30 minutes at room temperature **in the dark**.

    Note: TMB should not touch aluminum foil or other metals.



Add stop solution

13. Add 100 µL of Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.
4. Dilute samples producing signals greater than that of the highest standard in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0-4,000 pg/mL Hu PAI-1.

Standard Hu PAI-1 (pg/mL)	Optical Density (450 nm)
4,000	3.07
2,000	1.97
1,000	1.31
500	0.60
250	0.38
125	0.25
62.5	0.18
0	0.08

Specificity

Buffered solutions of a panel of substances ranging in concentrations from 1,320 to 100,000 pg/mL were assayed with the Hu PAI-1 kit and found to have no cross-reactivity: **Human** Eotaxin, GM-CSF, IFN-α, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 p40/p70, IL-13, IL-15, IL-17, IL-23, IP-10, MCP-1, MIG, MIP-1a, MIP-1β, PAI-2, RANTES, Serpin A3; **Rat** GM-CSF, IFN- γ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, TNF-α; **Mouse** IL-1α, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17, IP-10, MIP-1α, KC, MCP-1, PAI-1, TNF-α, VEGF.

Sensitivity

The minimum detectable dose of Hu PAI-1 is <30 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times, and calculating the corresponding concentration.

Performance characteristics, continued

Intra-assay precision

Samples of known Hu PAI-1 concentration were assayed in replicates of 14 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	339.8	982.6	2414.9
SD	12.5	48.7	103.2
%CV	3.7	5.0	4.3

SD = Standard Deviation; CV = Coefficient of Variation

Inter-assay precision

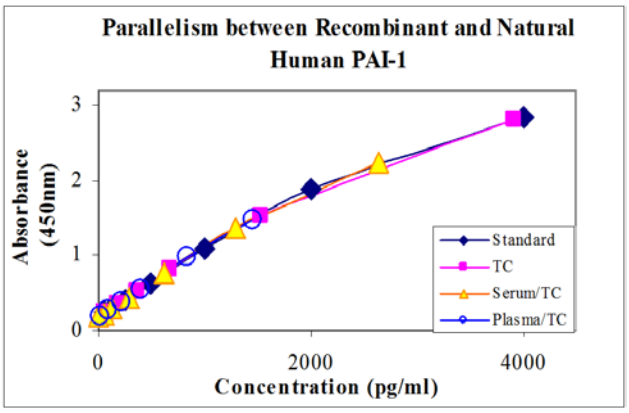
Samples were assayed 42 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	361.7	961.2	2466.6
SD	32.7	76.8	150.2
%CV	9.0	8.0	6.1

SD = Standard Deviation; CV = Coefficient of Variation

Parallelism

Random human serum, plasma and cell culture medium samples were serially diluted in the Standard Diluent Buffer and analyzed as described in the procedure. The optical density of each dilution was plotted against the Hu PAI-1 standard curve. Parallelism was demonstrated by the figure below and indicated that the standard accurately reflects the Hu PAI-1 content in natural samples.



Linearity of dilution

Human serum, EDTA plasma, citrate plasma, heparin plasma, spiked with tissue culture medium, and tissue culture medium were serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded average correlation coefficients of 0.999 for serum, 0.999 for EDTA plasma, 0.995 for citrate plasma, 0.992 for heparin plasma, and 0.986 for tissue culture medium.

Expected Values

Forty-one human serum and plasma samples, and unstimulated and PMA-stimulated HepG2 cell culture medium were evaluated for the presence of Hu PAI-1 in this assay.

Sample	Range (pg/mL)
Hu Serum (n=26)	320-8560
Hu EDTA plasma (n=5)	580-2600
Hu Heparin plasma (n=5)	180-1540
HepG2 cell culture medium, unstimulated	1930
HepG2 cell culture medium, stimulated with 100nM PMA	9810

Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

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