

IMUBIND[®] Tissue PAI-1 ELISA

REF 821

For Research Use Only



INTENDED USE

The IMUBIND[®] Tissue PAI-1 ELISA kit is an enzyme-linked immunoassay for the determination of human PAI-1 in tissue extracts and cell culture supernatants. The assay detects latent (inactive) and active forms of PAI-1 and PAI-1 complexes and is insensitive to PAI-2.

This assay is for research use only. It is not intended for diagnostic or therapeutic procedures.

EXPLANATION OF THE TEST

Plasminogen activator inhibitor type 1 (PAI-1), a primary regulator in fibrinolysis,^{1,2} has been found in a number of different cell types, i.e. macrophages/monocytes, platelets, vascular endothelial and adipose tissue of the heart and lungs.³ PAI-1 has been implicated along with urokinase-type plasminogen activator (uPA) as a prognostic factor for relapse-free and overall survival in breast cancer and gastric cancer patients. In patients diagnosed with primary breast cancer, PAI-1 ranks second to only lymph node status as a prognostic factor for Disease-free Survival (DFS) and Overall Survival (OS), with the established prognostic factors of node status, grading, tumor size and hormone receptor status.⁷ In ancillary node-negative patients, PAI-1 ranks highest with the established factors of tumor size, grading and hormone receptor status and experimental factors such as uPA (Urokinase Plasminogen Activator), Cathepsin D and S-Phase Fraction.⁸

It has been shown that a combination of PAI-1 and uPA further advances the delineation of patients having a low or high risk for relapse independent of the classical risk factors. Therefore by combining PAI-1 and uPA levels in tissue extracts a more individualized estimation of prognosis becomes possible in breast cancer patients.⁴⁻⁸ PAI-1 positivity may also serve as a prognostic factor for completely resected gastric cancer patients.⁹

PRINCIPLE OF THE METHOD

The IMUBIND Tissue PAI-1 ELISA employs a murine anti-human PAI-1 antibody as the capture antibody. Samples incubate in microwells coated with the monoclonal anti-human PAI-1 and are detected with a biotinylated anti-human PAI-1 antibody that recognizes the bound PAI-1 molecules. Adding streptavidin conjugated horseradish peroxidase (HRP) completes the formation of the antibody-enzyme detection complex.

The addition of a perborate/3, 3', 3, 5' - tetramethylbenzidine (TMB) substrate, and its subsequent reaction with HRP creates a blue colored solution. Sensitivity is increased by addition of a sulfuric acid stop solution, yielding a yellow color. PAI-1 levels are quantified by measuring solution absorbances at 450 nm and comparing the values with those of a standard curve.

REAGENTS

96 Anti-human PAI-1 IgG coated microwells (6, 16 well strips in a frame) and acetate cover sheet
 6 vials PAI-1 Standards, 0-10 ng/mL (lyophilized)
 2 vials Detection Antibody, biotinylated anti-human PAI-1 (lyophilized)
 1 vial Enzyme Conjugate, Streptavidin-horseradish peroxidase, 60 µL
 1 vial Enzyme Conjugate Diluent (lyophilized)
 1 vial Substrate, TMB, 11 mL
 1 vial Detergent, 25% Triton X-100, 12 mL
 2 packets PBS Buffer, pH 7.4

WARNINGS AND PRECAUTIONS

For Research Use only. Do not use kit components beyond the expiration date. Do not mix reagents from different kits. Do not smoke, eat or drink in areas where specimens or kit reagents are handled. Avoid microbial contamination of the kit components. Do not mouth pipette or ingest reagents.

PAI-1 standards	Warning		H319, P264, P280, P305+P351+P338, P337+P313
Detection antibody	Warning		H319, P264, P280, P305+P351+P338, P337+P313
Enzyme conjugate	Warning		CONT 2-Methyl-4-isothiazol-3-one H317, P261, P272, P280, P302+P352, P333+P313, P362
Enzyme conjugate Diluent	Danger		CONT Polyethylene glycol octylphenol ether H317, H318, H334, H412, P261, P280, P273, P305+P351+P338, P310
Detergent	Danger		CONT Polyethylene glycol octylphenol ether H318, H411, P280, P273, P305+P351+P338, P310
PBS packet	Warning		H319, P264, P280, P305+P351+P338, P337+P313

Hazard Statements:
 H317 May cause an allergic skin reaction.
 H318 Causes serious eye damage.
 H319 Causes serious eye irritation.
 H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
 H411 Toxic to aquatic life with long lasting effects.
 H412 Harmful to aquatic life with long lasting effects.

Precautionary Statements:
 P261 Avoid breathing dust.
 P264 Wash thoroughly after handling.
 P272 Contaminated work clothing must not be allowed out of the workplace.
 P273 Avoid release to the environment.
 P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
 P302 + P352 IF ON SKIN: Wash with plenty of water.
 P305 + P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
 P310 Immediately call a POISON CENTER/doctor.
 P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.
 P337 + P313 If eye irritation persists: Get medical advice/attention.
 P362 Take off contaminated clothing and wash before reuse.

REAGENT PREPARATION AND STORAGE

A. PAI-1 Standards

1. Add 1.0 mL filtered deionized water to the 1.0, 2.5, 5.0, 7.5 and 10.0 ng/mL standard vials and 2.0 mL filtered deionized water to the 0.0 ng/mL standard vial.
2. Agitate gently for 3 minutes. Do Not Shake!

B. Detection Antibody

Add 5.5 mL filtered deionized water per vial and agitate gently for 3 minutes.

C. Enzyme Conjugate Diluent

Add 20 mL filtered deionized water to the vial and mix well.

D. Wash Buffer

1. Dissolve contents of 1 PBS packet in 900 mL of filtered deionized water.
2. Add 4 mL of 25% Triton X-100 detergent.
3. Dilute to a final volume of 1 Liter with filtered deionized water.
4. Mix well.

E. Sample Buffer

Prepare an appropriate amount of Sample Buffer by adding BSA to Wash Buffer to a final concentration of 1% w/v (1 gm BSA to 100 mL Wash Buffer).

F. 10% Triton X-100

Add 4 mL of 25% Triton X-100 to 6 mL of filtered deionized water.

Store unused antibody coated microwells, liquid reagents and lyophilized reagents at 2°-8°C until the expiration dates indicated on labels. Reconstituted reagents may be stored at 2°-8°C for up to 2 weeks.

SPECIMEN COLLECTION AND PREPARATION

A. Detergent Extraction of Homogenized Tissue Samples

1. Suspend powder from homogenized frozen tissue samples (100-300 mg wet weight) in 1.8 mL TBS, pH 8.5.
2. Add 0.2 mL 10% Triton X-100 in TBS, pH 8.5, to the tissue suspension to yield a 1% Triton X-100 final preparation.
3. Stir for 16 hours at 4°C.
4. Centrifuge the suspension at 100,000 x g for 60 minutes at 4°C to separate cell debris.
5. Decant the supernatant/tissue extract and measure the total protein content of the extract using a BCA protein assay. If necessary, adjust the total protein content to 2-3 mg/mL with TBS, pH 8.5. Aliquot the extract into 100 µL portions.
- 6a. For storage, freeze at -80°C or in liquid nitrogen.
- 6b. For immediate use in the ELISA, dilute the tissue extract 1:20 in Sample Buffer.

B. Tissue Culture Supernatants

Dilute sample 1:5 (recommended initial dilution) in Sample Buffer. Note: some cell systems may require a higher dilution factor (up to 1:500).

PROCEDURE

Materials Provided – See Reagents

Materials Required But Not Provided

0.22 µm filtered deionized water
50-200 µL eight channel multi-pipette
10-200 µL single pipette
Microwell plate reader at 450 nm
0.5 M H₂SO₄ (*Note: Use caution when handling sulfuric acid*)
Bovine Serum Albumin (BSA, e.g. Sigma A-7030)
TRIS Buffered Saline (TBS), pH 8.5

Assay Procedure

Day One

1. Remove the necessary number of antibody coated microwells from the foil pouch. Return unused microwells to the pouch and reseal it with the desiccant inside and store at 2°-8°C.
2. Add 100 µL of PAI-1 Standard, control or diluted sample to the microwell, cover and incubate 16-20 hours at 4°C. Perform measurements in duplicate.

Day Two

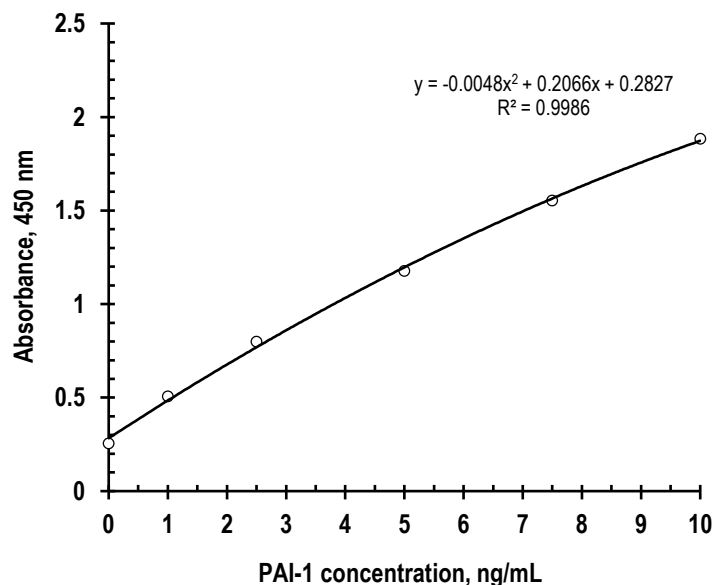
3. Wash all microwells 4 times with Wash Buffer.
4. Add 100 µL of Detection Antibody to each microwell, cover and incubate for 1 hour at room temperature.
5. Wash all microwells 4 times with Wash Buffer.
6. For using all 96 microwells at one time, add 12 µL of Enzyme Conjugate to 12 mL of Enzyme Conjugate Diluent (add 2 µL conjugate to 2 mL of diluent for each 16 well strip when using less than 96 wells). Add 100 µL of diluted enzyme conjugate to each microwell, cover and incubate for 1 hour at room temperature.
7. Wash all microwells 4 times with Wash Buffer.
8. Add 100 µL of Substrate solution to each microwell, cover and incubate for 20 minutes at room temperature. A blue color will develop.
9. Stop the enzymatic reaction by adding 50 µL of 0.5 M H₂SO₄. Tap the sides of the microwells to ensure even distribution of the H₂SO₄. The solution color will turn yellow. Read the absorbances on a microwell plate reader at a wavelength of 450 nm within 10 minutes.

RESULTS

Representative Standard Curve

Construct a standard curve by plotting the mean absorbance value calculated for each PAI-1 standard versus its corresponding PAI-1 concentration. Interpolate the PAI-1 concentrations for the diluted samples directly from the standard curve. A standard curve should be generated each time the assay is performed. The following curve is for demonstration purposes only. It has been plotted using a 2nd order polynomial regression analysis.

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Calculations

Using the mean absorbance value for each diluted sample, determine the corresponding PAI-1 concentration in ng/mL obtained from the standard curve.

A. Tissue Samples

1. Multiply the sample value by 20 to obtain the PAI-1 concentration of the tissue extract as the extract was diluted 20-fold in the assay.
2. Divide the PAI-1 concentration by the protein concentration (mg/mL) of the tissue extract to convert ng PAI-1/mL of sample to ng PAI-1/mg total protein.

B. Tissue Culture Supernatants

Multiply the sample value by the dilution factor to determine the PAI-1 concentration of the tissue culture supernatant.

REFERENCES

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4. Jänicke, F., *et al.* *Seminars in Thrombosis & Hemostasis* 1991; **17**: 303-312.
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DEFINITION OF SYMBOLS

	Consult instructions for use
	Refer to SDS
	Manufactured by
	Store at 2°C to 8°C
	Batch code / Lot number
	Expiration Date
	Catalog number
	Contains sufficient for <n> tests
	contains