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IMUBIND® PAI-2 ELISA

Product no. ADG823

Storage: 2 – 8°C **For Research Use Only!**

PRODUCT INSERT ***ENGLISH***

INTENDED USE

The IMUBIND® PAI-2 ELISA is an enzyme-linked immunoassay for the determination of human PAI-2 in human biological fluids.
This assay is for research use only. It is not intended for diagnostic or therapeutic procedures.

EXPLANATION OF THE TEST

This assay detects the low molecular weight (48 kD) and the high molecular weight glycosylated (60 kD) form of PAI-2. Free PAI-2 and PAI-2/uPA and PAI-2/tPA complexes are recognized. The assay is insensitive to PAI-1.

PRINCIPLE OF THE METHOD

Diluted samples are added to microwells coated with a polyclonal antibody against human PAI-2. During an incubation period, PAI-2 present in the sample will bind to the antibody coated to the wells. Following a washing step, a biotinylated monoclonal anti-PAI-2 antibody is added to the microwells and binds to the PAI-2 protein captured on the plate during a short incubation period. A streptavidin-horseradish peroxidase conjugate (SA-HRP) is added to the microwells to complete the formation of the antibody-enzyme detection complex. Following another washing step, the addition of a perborate-3,3'-5,5'-tetramethylbenzidine (TMB) substrate and its subsequent reaction with the HRP present generates a blue colored solution. The reaction is stopped by adding citrate stop solution, which turns the solution color yellow. Measuring the solution absorbance at 450 nm and extrapolating the value with those of a standard curve determines the level of PAI-2 in the samples.

REAGENTS

- **MTP** **Antibody Coated Microtiter plate,** MTP-96 (12x8) well
- **WASH** **Wash buffer**, 50 ml, 1 vial (20x concentrate)
- **DILB** **Dilution buffer**, 50 ml, 1 vial (ready-to-use)
- **STD** **Standard,** PAI-2, 5 ng/ml, 1 ml, 1 vial (lyophilized)
- **AB** **Detection antibody**, biotinylated mAb, 120 µl, 1 vial (100x concentrate)
- **CON** **Enzyme conjugate**, SA-HRP, 120 µL, 1 vial (100x concentrate)
- **TMB** **Substrate**, 11 ml, 1 vial (ready-to-use)
- **STOP** **Stop solution**, 6 ml, 1 vial (ready-to-use)

PRECAUTIONS

Not for internal use in humans or animals. Do not use the kit components beyond the expiration date. Do not mix reagents from different kit lots. Avoid microbial contamination of the reagents. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Do not pipette reagents by mouth or ingest reagents. Wear laboratory coat and disposable gloves throughout the test procedure and wash hands thoroughly afterwards. Handle gently; avoid splashing, foam, or aerosol formation.

WASH DILB AB CON	Warning		H317, P280, P333+P313
STD	Warning		H315, H317; H319, P280, P305+P351+P338, 337+P313, P302+P352, P333+P313
STOP	Warning		H314, P303+P361+P353, P305+P351+P338, P310

Hazard Statements:
H314 Causes severe skin burns and eye damage
H315 Causes skin irritation.
H317 May cause an allergic skin reaction.
H319 Causes serious eye irritation.

Precautionary Statements:
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection
P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention.
P303 + P361 + P353 IF ON SKIN (or hair): Remove/ Take off immediately all contaminated clothing. Rinse skin with water/shower.
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.
P337 + P313 If eye irritation persists: Get medical advice/attention.
P310 Immediately call a POISON CENTRE or doctor/physician.

REAGENT PREPARATION AND STORAGE

Unopened and lyophilized reagents are stable until the expiration date printed on the box when stored as instructed.

MTP **Antibody coated microwells:** Once removed from the foil pouch, the microwell strips must be used within 30 minutes. Unused strips may be stored at 2°-8°C for 4 weeks when sealed in the original pouch with the desiccant present, protected from any moisture.

WASH **Wash buffer:** If crystals are visible, incubate the vial in a 37°C water bath a few minutes until the crystals are dissolved. Transfer the content to a 1 liter bottle and fill up the concentrate to 1 liter with filtered deionized/distilled water. Diluted Wash Buffer may be used for up to 3 month when stored at 2°-8°C.

DILB **Dilution buffer:** Supplied ready to use. Opened sample buffer is stable for 3 month when stored at 2°-8°C.

STD **Standard:** Reconstitute the standard with 1 ml purified, deionised or distilled water, swirl the contents gently and allow the vials to stand at room temperature for at least 15 minutes to ensure complete dissolution. The lyophilised plasma is stable until the date indicated on the vial label when stored at 2° - 8°C. Once reconstituted, the plasma will remain stable for 3 month when stored at -20 °C.

AB **Detection antibody:** Supplied as a concentrate, dilute the Detection antibody 1:100 with Dilution buffer just prior to use. For using all 96 microwells at one time, dilute 100 µL of Detection Antibody to 10 mL in Dilution buffer. If not all 96 microwells are used, dilute 10 µL of Detection antibody to 1 mL in Dilution buffer for each 8-microwell strip that will be used. Working strength Detection antibody is stable for 4 hours at 2°-8°C. Discard any unused working strength Detection antibody. Opened antibody is stable for 3 month when stored in the dark at 2°-8°C.

CON **Enzyme conjugate:** Supplied as a concentrate, dilute the Enzyme conjugate 1:100 with Dilution buffer just prior to use. For running all 96 microwells at one time, dilute 100 µL of Enzyme conjugate to 10 mL in Dilution buffer. If not all 96 microwells are used, dilute 10 µL of Enzyme conjugate to 1 mL in Dilution buffer for each 8-microwell strip that will be used. Working strength Enzyme conjugate is stable for 2 hours at 2°-8°C. Discard any unused working strength Enzyme conjugate.

TMB **Substrate, TMB:** Supplied ready to use. Opened substrate is stable for 3 month when stored in the dark at 2° - 8°C.

STOP **Stop solution:** Supplied ready to use. Opened stop solution is stable for 3 month when stored at 2° - 8°C.

SPECIMEN COLLECTION AND PREPARATION

SAMPLE PREPARATION

A. Plasma

Nine volumes of blood are collected in 1 volume of 0.1M trisodium citrate or 99 volumes of blood in 1 volume of 0.5M EDTA, followed by centrifugation at 2500 X g for 15 minutes. See "Collection, Transport and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays", NCCLS Document H21-A2, Vol. 11, No. 23, 1991.

Plasma should be stored at 2°-8°C and assayed within 2 hours. Alternatively, plasma may be stored at -70°C for up to 2 years and then thawed once at 37°C, 30 minutes before use.

Dilute the plasma 1:50 or 1:100 in Dilution Buffer, depending on the expected PAI-2 content. Pregnancy plasma with high PAI-2 content is preferably tested at a dilution of 1:100.

B. Human Tissue Samples

1. Suspend powder from pulverized 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 12 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 PBS, 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 Dilution Buffer.

PROCEDURE

Materials Provided – See Reagents

Material Required But Not Provided

0.22 µm filtered deionized H_2O
Tris Buffered Saline (TBS), pH 8.5
10% Triton X-100 (v/v) in TBS, pH 8.5
50-300 µL eight channel multi-pipette
0-200 µL, 200-1000 µL single pipettes
microwell plate reader for reading absorbance at 450 nm
microwell plate washer (optional), microwell plate shaker (optional)

Preparing the PAI-2 Standards

1. Reconstitute the PAI-2 Standard as instructed under REAGENT PREPARATION.
2. Make serially dilutions of the (1) PAI-2 Standard with Dilution buffer as follows. Use Dilution buffer as the 0% standard.

Tube		Dilution buffer	PAI-2 conc. [ng/ml]
(1)	250 µl reconst. PAI-2 standard	0 µl	5
(2)	250 µl from (1)	250 µl	2.5
(3)	250 µl from (2)	250 µl	1.25
(4)	250 µl from (3)	250 µl	0.6
(5)	250 µl from (4)	250 µl	0.3
(6)	250 µl from (5)	250 µl	0.15
(7)	-	250 µl	0

Running standard and samples in duplicate is recommended.

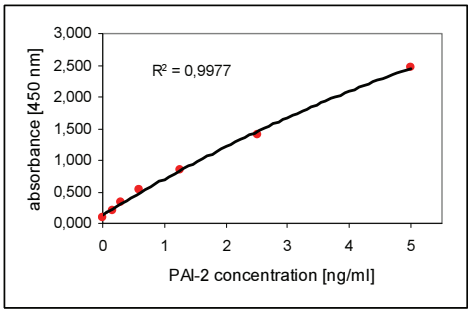
Assay Procedure

4. Open the foil pouch and remove the microwell strips/frame assembly. Remove the strips that will not be used, return them to the foil pouch and tightly reseal the pouch with the desiccant inside. Store the foil pouch at 2 - 8°C.
5. Pipette 100 µL of the standard, or diluted samples into separate microwells, cover with the acetate sheet and incubate for 1 hour at room temperature (18-25°C) on an orbital microwell plate shaker with agitation (at 250 rpm).
6. Empty the contents of the microwells and wash 4 times with Wash Buffer. Washing may be performed either using microwell plate washing equipment or manually (fill the wells with Wash Buffer with a pipette or squeeze bottle, wait three minutes, empty and remove droplets by tapping the plate 4-5 times face down against absorbing material).
7. Add 100 µL working strength Detection antibody to each microwell, cover with the acetate sheet and incubate the wells at room temperature (18-25°C) for 1 hour on an orbital microwell plate shaker with agitation (at 250 rpm).
8. Wash the wells by repeating Step 6.
9. Add 100 µL of working strength Enzyme conjugate to each microwell, cover with the acetate sheet and incubate for 1 hour at room temperature (18-25°C) on an orbital microwell plate shaker with agitation (at 250 rpm).
10. Wash the wells by repeating Step 6.
11. Add 100 µL of Substrate to each microwell immediately after the wash step, cover the wells with the acetate sheet and incubate for 5-10 minutes at room temperature (18°-25°C). A blue color will develop.
12. Stop the enzymatic reaction by adding 50 µL Stop solution to each microwell. Add the stop solution with the same speed and order as you added the substrate. Tap the sides of the microwell frame to ensure even distribution of the solution. The solution color will turn yellow. Read the absorbance on a microwell plate reader at a wavelength of 450 nm within 10 minutes.

RESULTS

Construct a standard curve by plotting the mean absorbance value for each standard versus its corresponding concentration. A standard curve should be generated each time the assay is performed. The following standard curve is for demonstration purposes only.

Representative Standard Curve



CALCULATIONS

Using the mean absorbance value for each diluted sample, determine the corresponding PAI-2 concentration in ng/mL obtained from the standard curve.

A. Plasma

Multiply the calculated concentration by the dilution factor to determine the PAI-2 concentration of the original plasma sample.

B. Tissue Extracts

1. Multiply the sample value by the dilution factor to obtain the PAI-2 concentration of the original tissue extract.

2. Divide the PAI-2 concentration of the tissue extract (ng/mL) by the protein concentration of the tissue extract (mg/mL) to convert ng PAI-2/mL of sample to ng PAI-2/mg protein.

EXPECTED VALUES

Generally, PAI-2 is undetectable in the majority of peripheral blood samples. However high levels of PAI-2 were foung in cytosol of primary breast tumors and in blood and tumor fluids of patients with ovarian cancer.[1-3] PAI-2 levels appear to increase during pregnancy.[5-7]

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