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Human PAI-1/uPA Complex Antigen ELISA Kit

Catalog # HPAIUPAKT-COM

Strip well format. Reagents for up to 96 tests.

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INTENDED USE

Human PAI-1/uPA complex assay is intended for the quantitative determination of the covalent complex of uPA and its inhibitor PAI-1 in human plasma, serum, urine, tissue extracts, or cell culture media. **For research use only.**

BACKGROUND

Urokinase plasminogen activator (uPA) is a serine protease that activates plasminogen to plasmin in the blood fibrinolytic system. It is also implicated in events related to cell invasion/migration [1]. Plasminogen activator inhibitor type 1 (PAI-1) is involved in the regulation of the blood fibrinolytic system and forms a 1:1 covalent complex with uPA and tPA.

ASSAY PRINCIPLE

Human uPA in samples will bind to the capture antibody coated on the microtiter plate. Free and complexed enzyme will react with the capture antibody on the plate. After appropriate washing steps, polyclonal anti-human PAI-1 primary antibody binds to PAI-1/uPA complex captured on the plate. Excess antibody is washed away and bound polyclonal antibody is then reacted with the secondary antibody conjugated to horseradish peroxidase. TMB substrate is used for color development at 450nm. A standard calibration curve of PAI-1/uPA complex is prepared along with the samples to be measured. Color development is proportional to the concentration of PAI-1/uPA complex in the samples. Free uPA and PAI-1 will not be detected by this assay.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human uPA antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human PAI-1/uPA complex standard:** 1 vial lyophilized standard
- **Anti-human PAI-1 primary antibody:** 1 vial lyophilized polyclonal antibody
- **Anti-rabbit horseradish peroxidase secondary antibody:** 1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water.

SAMPLE COLLECTION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 200ng/ml standard solution.

Dilution table for preparation of human PAI-1/uPA complex standard:

PAI-1/uPA complex concentration (ng/ml)	Dilutions
100	500µl (BB) + 500µl (from vial)
50	500µl (BB) + 500µl (100ng/ml)
20	600µl (BB) + 400µl (50ng/ml)
10	500µl (BB) + 500µl (20ng/ml)
5	500µl (BB) + 500µl (10ng/ml)
2.5	500µl (BB) + 500µl (5ng/ml)
1	600µl (BB) + 400µl (2.5ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.25	500µl (BB) + 500µl (0.5ng/ml)
0.1	600µl (BB) + 400µl (0.25ng/ml)
0	500µl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100µl PAI-1/uPA complex standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures PAI-1/uPA complex in the 0.1-100ng/ml range. If the unknown is thought to have high complex levels, dilutions may be made in blocking buffer.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 2µl of conjugated secondary antibody in 10ml of blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

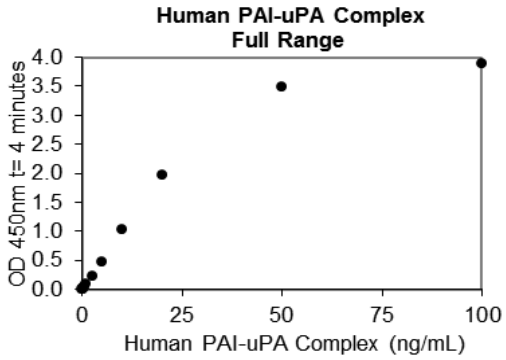
Measurement

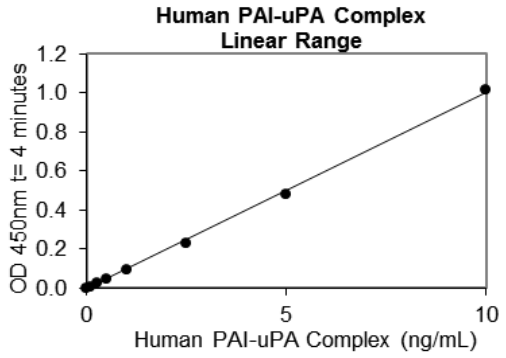
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of PAI-1/uPA complex in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of PAI-1/uPA complex in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):





EXPECTED VALUES

Concentrations of PAI-1/uPA complex in normal human plasma are low [2,3]. The median concentration of complex in plasma samples from breast cancer patients was 0.068ng/ml (n=18, range= <0.016-8.7ng/ml) [3]. Complex concentration in primary breast cancer tumor tissue extracts varied from 0.22–5.3ng/mg total protein (n=341, median=0.75ng/mg) and were prognostic for decreased tumor size and grade and increased survival [4]. Levels were associated with adverse grade and poor survival in a separate study [5]. Values for lung cancer tumor tissue extracts were similar to breast cancer and varied from 0.11—5.74ng/mg total protein (n=99, median=0.79ng/mg) [6].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD_{450}: 0.05-0.06) and calculating the corresponding concentration. The MDD was 0.070ng/ml.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Specificity: These studies are currently in progress. Please contact us for more information.

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

1. Kjøller L: Biol Chem. 2002, 383:5-19.
2. Grebenchtchikov N, *et al.*: J Immunol Meth. 2002, 268:219–231.
3. Pedersen AN, *et al.*: Clin Chem. 1999, 45:1206–1213.
4. Pedersen AN, *et al.*: Cancer Res. 2000, 60:6927–6934.
5. Manders P, *et al.*: Cancer. 2004, 101(3):486-494.
6. Pappot H, *et al.*: Lung Cancer. 2006, 51(2):193-200.

Example of ELISA Plate Layout
96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.1 ng/ml	0.25 ng/ml	0.5 ng/ml	1 ng/ml	2.5 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	
B	0	0.1 ng/ml	0.25 ng/ml	0.5 ng/ml	1 ng/ml	2.5 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	
C												
D												
E												
F												
G												
H												