Catalog # HPAIUPA-COM

Human PAI-1 uPA Complex Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

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 biological fluids.

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 is prepared along with the samples to be measured using dilutions of CPAI uPA complex. 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

- **Coated plate:**
  1-96 well immulon plate coated, blocked, and dried with capture antibody
- **10X Wash Buffer:**
  1 bottle of 50ml wash buffer; bring to 1X using DI water
- **Human PAI-1 uPA complex standard:**
  1 vial of lyophilized standard
- **Anti-human PAI-1 primary antibody:**
  1 vial of lyophilized rabbit polyclonal antibody
- **Anti-rabbit horseradish peroxidase conjugate secondary antibody:**
  1 vial concentrated HRP labeled antibody
- **TMB substrate solution:** 10 ml

STORAGE AND STABILITY

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

REAGENTS AND EQUIPMENT REQUIRED

- 1-channel pipettes covering 20-200 µl, 500-5000 µl and 200-1000µl
- 12-channel pipette for 30-300µl
- Paper towels or kimwipes
- 1.5ml micro centrifuge tubes
- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- TBS buffer
- Blocking buffer

•Microtiter plate spectrophotometer operable at 450nm
•Microtiter plate shaker with uniform horizontally circular movement up to 300rpm

WARNINGS

Warning – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

PRECAUTIONS

- **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.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:**
  0.10M TRIS, 0.15M NaCl, pH 7.4
- **Blocking buffer (BSA):**
  3% BSA in TBS buffer

SPECIMEN PREPARATION

Samples of human plasma, serum, urine, cell culture media, or tissue extracts may be applied directly to the plate.

The assay measures PAI-1 uPA complex in the 0.1-100 ng/ml range. Samples with complex levels above 100 ng/ml should be diluted in plasma or similar fluid devoid of PAI-1 or uPA.

ASSAY PROCEDURE

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

Preparation of Standard:
Reconstitute standard vial with 5ml of 3% BSA blocking buffer to give a 250 ng/mL standard solution.

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

Complex concentration (ng/mL)	Dilutions
100	600µL (BSA) + 400µL (250ng/mL)
50	500µL (BSA) + 500µL (100ng/mL)
20	600µL (BSA) + 400µL (50ng/mL)
10	500µL (BSA) + 500µL (20ng/mL)
5	500µL (BSA) + 500µL (10ng/mL)
2.5	500µL (BSA) + 500µL (5ng/mL)
1.0	600µL (BSA) + 400µL (2.5ng/mL)
0.5	500µL (BSA) + 500µL (1.0ng/mL)
0.25	500µL (BSA) + 500µL (0.5ng/mL)
0.1	600µL (BSA) + 400µL (0.25ng/mL)

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

Standard and Unknown Addition:
Remove microtiter plate from bag. Add 100µl standard in duplicate and unknown 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.

Primary Antibody Addition:
Add 10ml of 3% BSA blocking buffer directly to the primary antibody 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.

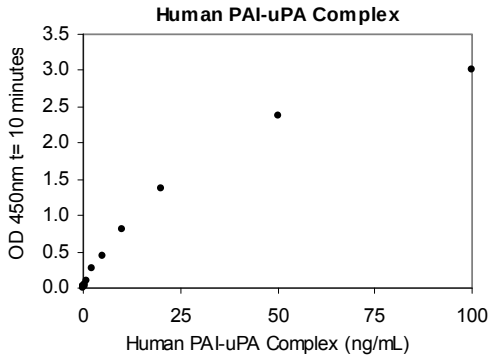
Secondary Antibody Addition:
Dilute 2.5µl into 10ml of 3% BSA blocking buffer and add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

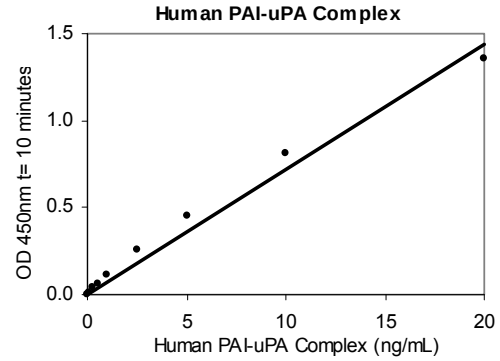
Substrate Incubation:
Add 100µl TMB substrate to all wells and shake plate for 5-15 minutes. Quench the reaction by the addition of 50µl of 1M H_2SO_4 and read final absorbance values at 450nm.
NOTE: Time for substrate development is dependent on needs of researcher.

Measurement:
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm, A_{450}.

Assay Calibration:
Plot A_{450} against the amount of PAI-1 uPA complex in the standards. Fit a straight line through the points using a linear fit procedure. The PAI-1 uPA complex concentration in the unknowns can be determined by from this curve.

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





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 0.068 ng/ml (n=18, range= <0.016-8.7 ng/ml) [3]. Complex concentration in primary breast cancer tumor tissue extracts varied from 0.22–5.3 ng/mg total protein (n=341, median=0.75 ng/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—5.74 ng/mg total protein (n=99, median=0.79 ng/mg) [6].

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 or from contact with the above product.

REFERENCES

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3. Pedersen AN et al.: Determination of the Complex between Urokinase and Its Type-1 Inhibitor in Plasma from Healthy Donors and Breast Cancer Patients. Clin. Chem., **45**: 1206 – 1213, 1999.
4. Pedersen AN et al.: The Complex between Urokinase and Its Type-1 Inhibitor in Primary Breast Cancer: Relation to Survival. Cancer Res., **60**: 6927 – 6934, 2000.
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