Enzyme Immunoassay for
Human Urokinase Plasminogen Activator (uPA) Total Antigen

For Research Use Only

INTRODUCTION

Human uPA total antigen assay is intended for the quantitative determination of total plasminogen activator antigen in biological fluids.

Urokinase plasminogen activator 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).

PRINCIPLES OF PROCEDURE

Human uPA 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 uPA primary antibody binds to the captured enzyme. 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 450 nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of uPA.

MATERIALS PROVIDED

Component	Contents	Quantity	Storage	Cat. No.
Coated Plate	Capture antibody coated 96-well plate	1 plate	4°C	UP40a
Standard	Human uPA activity standard	1 vial	4°C	UP40b
Primary Antibody	Rabbit anti-human uPA antibody	1 vial	4°C	UP40c
Wash Buffer	10x solution for washing plate	50 mL	4°C	UP40d
Substrate	TMB substrate	10 mL	4°C	UP40e
Secondary Antibody	Anti-rabbit HRP conjugated antibody	1 vial	4°C	UP40f

MATERIALS NEEDED BUT NOT PROVIDED

1. Pipettes covering 0-10 μl and 200-1000 μl and tips
2. 12-channel pipette covering 30-300 μl
3. 1N H_2SO_4
4. DI water
5. Microtiter plate spectrophotometer with a 450 nm filter
6. Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm

STORAGE CONDITIONS

1. Store this kit and its components at 4°C until use.
2. 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.

PROCEDURAL NOTES

1. Use aseptic technique when opening and dispensing reagents.
2. This kit is designed to work properly as provided and instructed. Additions, deletions, or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.
3. Exercise universal precautions during the performance or handling of this kit or any component contained therein.

SAMPLE COLLECTION AND PREPARATION

Samples of human plasma, serum, urine, cell culture media, or tissue extracts may be applied directly to the plate. The assay measures total uPA in the 0.1-50 ng/ml range. Samples giving uPA levels above 50ng/ml should be diluted in plasma or similar fluid devoid of uPA.

REAGENT PREPARATION

1. Dilute the 50 mL of 10x Wash Buffer concentrate to 1x with 450 mL of DI water prior to use.
2. Prepare 100 mL of TBS Buffer: 0.1 M Tris-HCL, 0.15 M NaCl, pH 7.4
3. Prepare 20 mL of 3% BSA Blocking Buffer: 3% BSA in TBS Buffer

STANDARD PREPARATION

Reconstitute the Standard according to the attached dilution table. **Do not prepare the Standards until you are ready to apply them to the plate.**

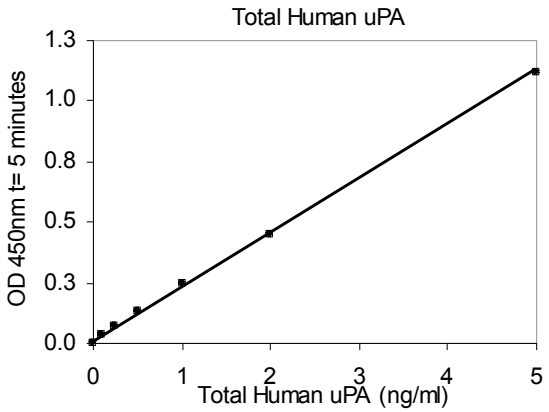
ASSAY PROCEDURE

1. Add 100 μl of the Standards and unknowns to the wells in duplicate.
2. Shake the plate at 300 rpm for 30 minutes at room temperature.
3. Wash the plate three times with 300 μL of Wash Buffer. Remove excess Wash Buffer by gently tapping the plate on a paper towel.
4. Reconstitute the Primary Antibody with 3% BSA Blocking Buffer as directed on the vial and agitate gently to completely dissolve contents. Add 100 µl to each well.
5. Shake the plate at 300 rpm for 30 minutes at room temperature.
6. Wash the plate three times with 300 μL of Wash Buffer. Remove excess Wash Buffer by gently tapping plate on a paper towel.
7. Dilute the Secondary Antibody in 3% BSA Blocking Buffer as directed on the vial and add 100 µl to each well.
8. Shake the plate at 300 rpm for 30 minutes at room temperature.
9. Wash the plate three times with 300 μL of Wash Buffer. Remove excess Wash Buffer by gently tapping the plate on a paper towel.
10. Add 100 μl of TMB Substrate to each well.
11. Shake the plate at 300 rpm for 2-10 minutes at room temperature.
12. Stop the reaction with 50 μl of 1N H_2SO_4 and read the plate at 450 nm.

CALCULATIONS

1. Plot the A_{450} against the concentration of uPA in the standards.
2. Fit a straight line through the points using a linear fit procedure.
3. Calculate the uPA concentrations in the unknowns using the standard curve. See Figure 1 on the following page for an example of a typical standard curve.

Figure 1: Typical Standard Curve
(Do Not Use For Calculations)



EXPECTED VALUES

The concentration of uPA antigen in human plasma has been reported to be 3.5 ± 1.4 ng/ml (mean ± SD, n = 54) (2). Culture media of fibroblasts, endothelial- and kidney cells showed antigen levels of 1.2, 23 and 65 ng/ml, respectively (2). The uPA antigen level in human bile is reported as 0.705 ng/ml (3).

Detergent extracts of human tumor tissues had the following uPA antigen levels (4):

Cancer	Mean	Range	n
Lung	6.28	(3.33–18.23)	6
Cervical	5.03	(2.33–8.72)	6
Colon	3.65	(2.30–8.80)	6
Melanoma	1.04	(0.62–1.77)	6
Breast	2.67	(0.00–8.96)	9

Abnormalities in uPA levels have been reported in the following conditions:

- Venous Thrombosis: Low levels of uPA are associated with clot formation (5).
- Inflammatory Disease: Low levels of uPA may aggravate this condition (6).

REFERENCES

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