

Human uPA Activity Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

Human uPA activity assay is intended for the quantitative determination of active urokinase plasminogen activator in human plasma.

BACKGROUND

Urokinase plasminogen activator, along with its receptor uPAR, initiates a proteolytic cascade that results in the conversion of plasminogen to plasmin [1]. Clinical studies have indicated that high uPA levels may elevate the risk for tumor invasion and metastasis [2,4]. Increased expression and activity can exert potent arthritogenic properties in rheumatoid arthritis patients [3]. Increased uPA activity may be an implication for the pathophysiology of endometriosis [5].

ASSAY PRINCIPLE

Functionally active uPA present in plasma reacts with the biotinylated human PAI-1 capture. Latent or complexed uPA will not bind to the plate and will not be detected. Unbound uPA samples are washed away and an anti-uPA primary antibody is added. Excess primary antibody is washed away and bound antibody, which is proportional to the original active uPA present in the samples, is then reacted with the horseradish peroxidase secondary antibody. Following an additional washing step, TMB is then used for color development at 450nm. The amount of color development is directly proportional to the concentration of active uPA in the sample.

REAGENTS PROVIDED

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

STORAGE AND STABILITY

All reagents 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 0-10µl and 200-1000µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 50ml tubes

- 1N H₂SO₄
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- TBS buffer
- 3% Blocking buffer
- Microtiter plate spectrophotometer operable at 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

WARNINGS

Warning - The uPA standards are of human origin. Each donor unit has been tested and found negative for the presence of HBsAg, anti-HIV 1+2, anti-HBc, and anti-HCV.

Since no tests are currently available to assure that no infectious agents are present, the standard must be treated as is recommended at the Biosafety Level 2 as potentially infectious human serum or blood specimen in the Centres for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories", 1984.

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:** 0.1M TRIS, 0.15M NaCl, pH 7.4
- **Blocking buffer (BSA):** 3% BSA in TBS buffer

SPECIMEN COLLECTION

Collect 9 volumes of blood in 1 volume of 0.1M trisodium citrate or acidified citrate, preferably using Stabilyte™ evacuated vials (Biopool, cat# 102080) [6]. This insures that the fast-acting inhibitor for high molecular weight uPA which is usually present in large excess, is inhibited from quenching uPA activity [5]. Immediately after collection of blood, samples must be centrifuged at 2500Xg for 15 minutes. The plasma must be transferred to a clean plastic tube and stored on ice prior to analysis. The uPA activity samples collected in the Stabilyte™ media is stable for up to 5 hours or frozen at -20°C for up to one month or up to 5 months at -70°C.

ASSAY PROCEDURE

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

Biotinylated Human PAI-1 Addition:

Remove microtiter plate from bag. Add 10ml 3% BSA blocking buffer directly to the biotinylated human PAI-1 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.

Preparation of Standard:

Prepare the uPA standard according to the dilution table.

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

Reconstitute vial with 1 ml of BSA, making Human uPA: 500ng/ml

uPA concentration (ng/ml)	Dilutions
50	900µl (BSA) + 100µl (vial)
20	600µl (BSA) + 400µl (50ng/ml)
10	500µl (BSA) + 500µl (20ng/ml)
5	500µl (BSA) + 500µl (10ng/ml)
2	600µl (BSA) + 400µl (5ng/ml)
1	500µl (BSA) + 500µl (2ng/ml)
0.5	500µl (BSA) + 500µl (1ng/ml)
0.25	500µl (BSA) + 500µl (0.5ng/ml)
0.1	600µl (BSA) + 400µl (0.25ng/ml)
0	500µl (BSA) Zero point to determine background

Standard and Unknown Addition:

Add 100µl uPA standards (enough for duplicates) and 100µl unknowns to wells. Carefully record the 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 active uPA in the 0.1-50 ng/ml range. If the unknown is thought to have high uPA levels, dilutions may be made in 3% BSA blocking buffer.

Primary Antibody Addition:

Reconstitute primary antibody by adding 10ml blocking buffer to vial. 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 conjugated secondary antibody in 10ml 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 of substrate solution to all wells and shake plate for 2-10 minutes. Quench the reaction with the addition of 50µl of 1N H₂SO₄ and read final absorbance values at 450nm.

NOTE: Time for substrate development is dependent on needs of researcher.

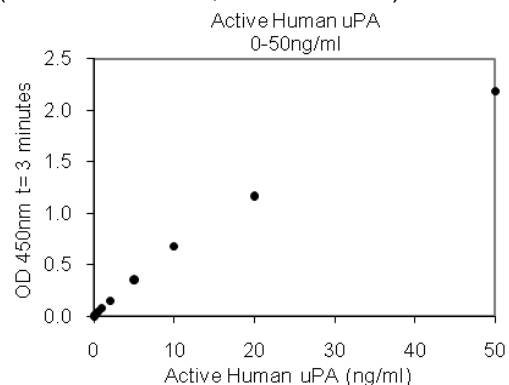
Measurement:

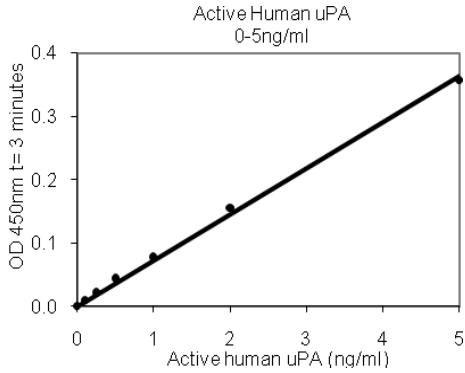
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A₄₅₀).

Assay Calibration:

Plot A₄₅₀ against the amount of uPA in the standards. Fit a straight line through the points using a linear fit procedure. The uPA activity in the unknowns can be determined from this curve.

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





EXPECTED VALUES

The mean value of uPA in healthy donors was found 1.1 +/- 0.3 ng/ml [6].

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

- ◆ **Breast Cancer:** Increased levels of uPA have been reported in patients with breast cancer compared to healthy donors [6].
- ◆ **Liver Cancer:** The detection of liver cancer increased when combining the information of two tumor markers, uPA and alpha-fetoprotein [7].
- ◆ **Pancreatic Cancer:** Increased uPA levels may serve as a prognostic marker in human pancreatic cancer [8,9].

PERFORMANCE CHARACTERISTICS

Sensitivity = 0.064 ng/ml

(calculated by determining the OD of 20 reps of S_0 and 20 reps of the low standard)

Linearity

The slope = 1.0589

Correlation coefficient = 0.9991

Intra Assay Precision

High 5.2%, Medium 2.5%, Low 5.7%
(calculated by running 20 reps of each concentration in an assay)

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.

REFERENCE

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6. Grondahl-Hansen J, *et al.*: Sensitive and specific enzyme-linked immunosorbent assay for urokinase-type plasminogen activator and its application to plasma from patients with breast cancer. J Lab Clin Med, Jan;**111(1)**: 42-51, 1988.
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