Catalog # MUPAKT

Mouse uPA Activity Assay

Strip well format. Reagents for up to 96 tests.

For Research Use Only.

INTENDED USE

Murine uPA activity assay is intended for the quantitative determination of active plasminogen activator in mouse plasma.

BACKGROUND

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 [3].

ASSAY PRINCIPLE

Functionally active uPA will bind to the biotinylated human PAI-1 coated on the microtiter plate. Only free active enzyme will react with the PAI-1 on the plate. Inactive or complexed enzyme will not be detected. After appropriate washing steps, monoclonal mouse anti-murine uPA primary antibody binds to the captured enzyme. Excess antibody is washed away and bound monoclonal 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 uPA.

REAGENTS PROVIDED

♦ **96-well plate (8X12 removable strips) coated microtiter strip plate:** containing avidin, dried and blocked on the strip well surface.

♦ **10X Wash Buffer:**
1 bottle of 50ml wash; bring to 1X using DI water

♦**Biotinylated PAI-1**: 1 vial lyophilized biotinylated PAI-1

♦**10X TBS Buffer pH 7.4:**
1 vial of 5ml TBS buffer

♦**Murine uPA activity standard:**
1 vial lyophilized activity standard

♦**Anti-murine uPA primary antibody:**
1 vial lyophilized primary antibody

♦**Anti-rabbit horseradish peroxidase conjugate secondary antibody:** 1 vial

♦**TMB substrate solution:**
1 bottle 10 ml solution

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 0-10µl and 200-1000µl
•12-channel pipette for 30-300µl
•Paper towels or kimwipes
•50ml tubes

- 1N H_2SO_4 (Stop Solution)
- 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 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). Immediately after collection of blood, samples must be centrifuged at 3000Xg for 15 minutes. It is important to ensure a platelet free preparation as platelets can release PAI-1, which in turn could potentially form a complex with uPA. The plasma must be transferred to a clean plastic tube and must be stored on ice prior to anaylsis. The uPA activity samples collected in the Stabilyte media are stable for up to 24 hours or stored at –20°C for up to one month and thawed three times without loss of uPA activity. If using kidney extracts that have been extracted using triton X, dialyze to remove the triton X before using in the assay. Detergents such as triton X may interfere with the assay.

ASSAY PROCEDURE

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

Preparation of Wash Buffer:

Dilute 50ml of the 10X wash buffer using 450ml DI water to make a 1X wash buffer solution.

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:

Reconstitute standard as directed on vial and agitate gently to completely dissolve contents.

Mouse uPA: 100ng/ml before reconstitution

uPA concentration (ng/ml)	Dilutions
10	900µl (BSA) + 100µl (100ng/ml=vial)
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)
0.05	500µl (BSA) + 500µl (0.1ng/ml)
0.025	500µl (BSA) + 500µl (0.05ng/ml)
0	500µl (BSA) Zero point to determine background

Samples giving uPA levels above 10ng/ml should be diluted in 3% BSA blocking buffer.

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

Standard and Unknown Addition:

If using citrated plasma or samples with pH lower than 6.0 add 30µl of 10X TBS buffer to each well. If using samples at a neutral pH this step should be omitted.

Add 100µl standard in duplicate and unknown to wells. 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: If the unknown is thought to have high uPA levels, dilutions may be made in 3% BSA blocking buffer.

Primary Antibody Addition:

Add 10ml 3% BSA 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 BSA 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 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 and read final absorbance values at 450nm. For best results read plate immediately

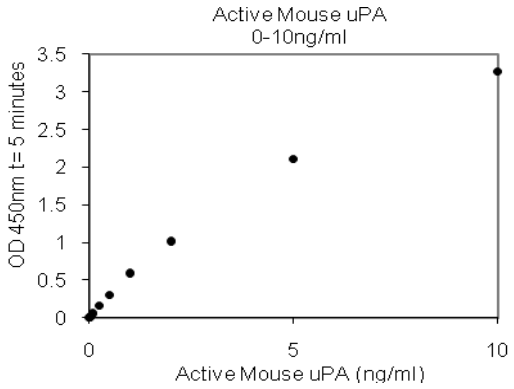
Measurement:

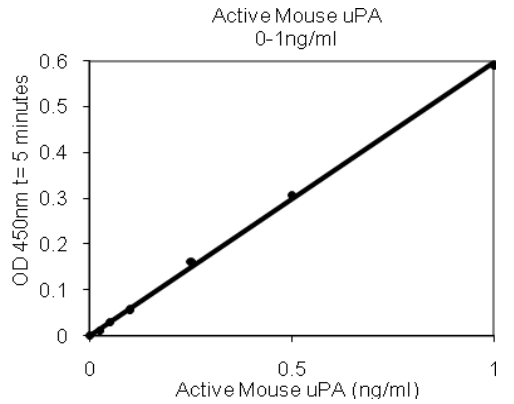
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Assay Calibration:

Plot A_{450} 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 by from this curve.

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





EXPECTED VALUES

The concentration level of uPA antigen in murine urine has been reported to be 1.8+/-1.9 ug/ml [1].

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

♦ Venous Thrombosis: Low levels of uPA is associated with clot formation [2].

♦ Inflammatory Disease: Low levels of uPA may aggravate this condition [4].

PERFORMANCE CHARACTERISTICS

Sensitivity = 0.004 ng/ml
(calculated by determining the OD of 20 reps of So and 20 reps of the low standard)

Linearity
The slope = 1.1062
Correlation coefficient = 0.9987

Intra Assay Precision
High 6.6%, Medium 5.9%, Low 4.4%
(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

1. Declerck PJ, *et al*.: Immunoassay of murine t-PA, u-PA, and PAI-1 using monoclonal antibodies raised in gene-inactivated mice. Thromb Haemostas., Nov **74(5**): 1305-9, 1995.

2. Singh I, *et al.*: Failure of thrombus to resolve in urokinase-type plasminogen activator gene-knockout mice: rescue by normal bone marrow-derived cells. Circulation, **107(6)**, 869-875, 2003.

3. Kjøller Lars: The Urokinase Plasminogen Activator Receptor in the Regulation of the Actin Cytoskeleton and Cell Motility. Biol. Chem., **383**: 5-19, 2002.

4. Yang YH, *et al*.: Tissue-type plasminogen activator deficiency exacerbates arthritis. J. Immunol., **167(2)**, 1047-52, 2001.

Example of Plate Layout:
96 well Plate,

Standards: 20 wells,
Samples: 76 wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.025ng/ml	0.05ng/ml	0.1ng/ml	0.25ng/ml	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml		
B	0	0.025ng/ml	0.05ng/ml	0.1ng/ml	0.25ng/ml	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml		
C												
D												
E												
F												
G												
H												