LSBio™ Mouse PLAU / Urokinase / UPA Enzyme Capture ELISA Kit

Catalog No. LS-F10471

User Manual

Please Read the Manual Carefully
Before Starting your Experiment

LSBio
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Mouse uPA Activity ELISA Kit

Catalog # LS-F10471 Strip well format. Reagents for up to 96 tests.

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INTENDED USE

Mouse uPA activity assay is intended for the quantitative determination of active plasminogen activator in mouse plasma. **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 [3].

ASSAY PRINCIPLE

Functionally active uPA will form a covalent complex with the biotinylated human PAI-1 which is bound to the avidin on the 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, anti-mouse uPA primary antibody binds to the captured enzyme. Excess antibody is washed away and bound antibody is then reacted with the secondary antibody conjugated to horseradish peroxidase. TMB substrate is 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

- **96-well avidin coated microtiter strip plate** (removable wells 8x12) containing avidin, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **10X TBS buffer, pH 7.4:** 1 bottle of 5mL
- **Biotinylated human PAI-1:** 1 vial lyophilized protein
- **Mouse uPA activity standard:** 1 vial lyophilized standard
- **Anti-mouse uPA 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 9 volumes of blood in 1 volume of 0.1M trisodium citrate or acidified citrate. 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 analysis. The uPA activity samples collected 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.

Biotinylated Human PAI-1 Addition

Add 10ml blocking buffer directly to the biotinylated human PAI-1 vial and agitate gently to completely dissolve contents. Remove microtiter plate from bag 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.

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 100ng/ml standard solution.

Dilution table for preparation of mouse uPA standard:

uPA concentration (ng/ml)	Dilutions
10	900µl (BB) + 100µl (from vial)
5	500µl (BB) + 500µl (10ng/ml)
2	600µl (BB) + 400µl (5ng/ml)
1	500µl (BB) + 500µl (2ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.25	500µl (BB) + 500µl (0.5ng/ml)
0.1	600µl (BB) + 400µl (0.25ng/ml)
0.05	500µl (BB) + 500µl (0.1ng/ml)
0.025	500µl (BB) + 500µl (0.05ng/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

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 uPA 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 active uPA antigen in the 0.025-10 ng/ml range. If the unknown is thought to have high uPA 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 2.5µl 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 5-15 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.

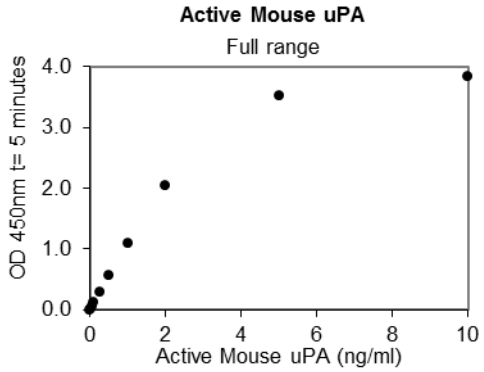
Measurement

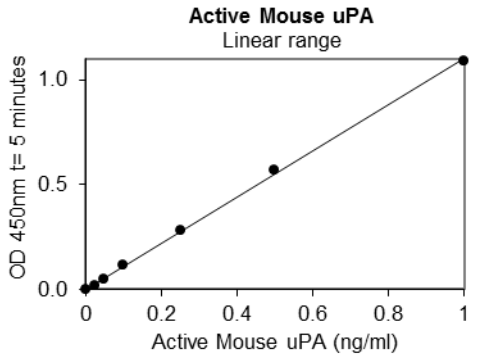
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of uPA 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 uPA 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

The concentration level of uPA antigen in murine urine has been reported as 1.8±1.9 µg/ml [1].

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

- 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: 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}: 0.063-0.086) and calculating the corresponding concentration. The MDD was 0.022 ng/ml.

Intra-assay Precision: Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	High	Medium	Low
n	20	20	20
CV (%)	6.6	5.9	4.4

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: slope = 1.1062,
correlation coefficient = 0.9987

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

Sample Values: Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean (µg/mL)
Balb/c Mouse Urine	1:4,000	0.298
	1:2,000	0.298
	1:1,000	0.286

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. Declerck PJ, *et al.*: Thromb Haemostas. 1995, 74(5):1305-9.
2. Singh I, *et al.*: Circulation. 2003, 107(6):869-875.
3. Kjøller L: Biol Chem. 2002, 383:5-19.
4. Yang YH, *et al.*: J Immunol. 2001, 167(2):1047-52.

Example of ELISA Plate Layout
96 Well Plate: 20 Standard wells, 76 Sample wells

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

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion.

Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

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