LSBio™ Mouse PLAU / Urokinase / UPA ELISA Kit

Catalog No. LS-F10453

User Manual

Please Read the Manual Carefully
Before Starting your Experiment

LSBio
LifeSpan BioSciences, Inc.

LSBio
LifeSpan BioSciences, Inc.
www.lsbio.com

Mouse uPA Total Antigen ELISA Kit

Catalog # LS-F10453 Strip well format.
Reagents for up to 96 tests.

Rev: April 2014

INTENDED USE

Mouse uPA total antigen assay is intended for the quantitative determination of total plasminogen activator antigen in mouse plasma. **For research use only.**

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 [1].

ASSAY PRINCIPLE

Mouse 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-murine 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 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of uPA.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-mouse uPA antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Mouse uPA 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 plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

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

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)
0.01	600µl (BB) + 400µl (0.025ng/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

Remove microtiter plate from bag and 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 uPA antigen in the 0.01-10 ng/ml range. If the unknown is thought to have high uPA levels, dilutions may be made in plasma devoid of uPA or 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 2-10 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.

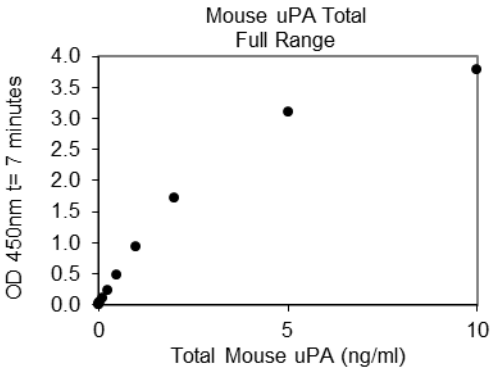
Measurement

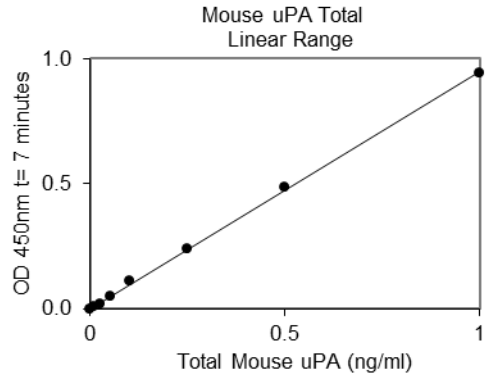
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

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 to be 1.8+/-1.9 ug/ml [2]. In house testing of pooled normal mouse plasma in citrate indicates uPA levels vary by mouse strain.

Strain	Total uPA
NSA/CF-1	2.6 ng/ml
C57BL6	1.4 ng/ml
CD-1	0.3 ng/ml

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

•Venous Thrombosis: Low levels of uPA is associated with clot formation [3].

•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}: 0.057-0.064) and calculating the corresponding concentration. The MDD was 0.00998 ng/ml.

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

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	0.06	0.30	3.01
Standard Deviation	0.005	0.019	0.112
CV (%)	8.12	6.18	3.73

Inter-assay Precision: Three samples of known concentration were tested in ten independent assays to assess inter-assay precision.

Sample	1	2	3
n	10	10	10
Mean (ng/ml)	0.09	0.25	2.66
Standard Deviation	0.008	0.019	0.154
CV (%)	8.52	7.47	5.79

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in blocking buffer was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/ml)	0.03	0.19	1.49	3.20
Average % Recovery	108	93	99	91
Range	92-115%	88-99%	94-103%	83-107%

Linearity: To assess the linearity of the assay, pooled mouse plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	101	96	102	105
Range	99-104%	92-103%	99-104%	103-106%

Specificity: This assay recognizes natural and recombinant total mouse uPA. Pooled normal plasma from rat and pig was assayed and significant cross-reactivity was observed. Pooled normal plasma from sheep was assayed and no significant cross-reactivity was observed. Pooled normal plasma from rabbit resulted in significant color development.

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

Sample Type	Dilution	Mean (ng/mL)
Citrate Plasma	Undiluted	6.82
	1:2	6.88

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

Example of ELISA Plate Layout
96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.01 ng/ml	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	0.01 ng/ml	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.

Returns, Refunds, Cancelations: Any problems with LifeSpan products must be reported to LifeSpan within 10 days of product receipt. The customer must obtain written authorization from LifeSpan before returning items. To request that goods be returned, please contact LifeSpan Technical Support. If an error by LifeSpan BioSciences results in shipment of an incorrect order, LifeSpan will, at its option, either ship a replacement order at no charge, or credit the customer's account for the original product shipped in error. Returns and cancelations may be subject to a 30% restocking fee.

Conditions & Warranty: All LifeSpan products are intended for Research Use Only and are not for use in human therapeutic or diagnostic applications. The information supplied with each product is believed to be accurate, but no warranty or guarantee is offered for the products, because the ultimate conditions of use are beyond LifeSpan's control. The information supplied with each product is not to be construed as a recommendation to use this product in violation of any patent, and LifeSpan will not be held responsible for any infringement or other violation that may occur with the use of its products. Under no event will LifeSpan be responsible for any loss of profit or indirect consequential damage, including, but not limited to, personal injuries resulting from use of these products. LifeSpan's liability to any user of Products for damages that do not result from any fault of the user, will be limited to replacement of the Product(s) only, and in no event shall LifeSpan's liability exceed the actual price received by LifeSpan for the Product(s) at issue. LifeSpan shall not be liable for any indirect, special, incidental or consequential damages. LIFESPAN FURTHER DISCLAIMS ANY AND ALL EXPRESS AND IMPLIED OR STATUTORY WARRANTIES WITH RESPECT TO THE PRODUCTS, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE. LifeSpan disclaims any and all responsibility for any injury or damage which may be caused by the fault of the user.

For research use only. Not approved for use in humans or for clinical diagnosis.

LSBio
LifeSpan BioSciences, Inc.

2401 Fourth Avenue Suite 900 Seattle, WA 98121
Tel: 206.374.1102
Fax: 206.577.4565

Technical.Support@LSBio.com