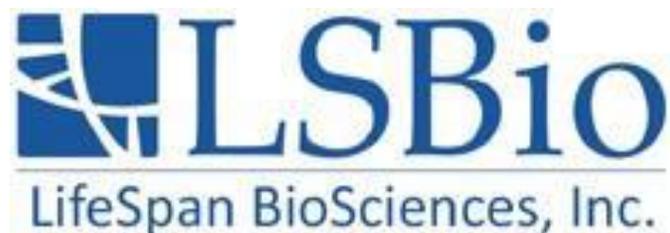


LSBio™ Mouse PLG / Plasmin / Plasminogen ELISA Kit

Catalog No. LS-F10445

User Manual

**Please Read the Manual Carefully
Before Starting your Experiment**



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



LifeSpan BioSciences, Inc.

Mouse Plasminogen Total Antigen ELISA Kit

Catalog # LS-F10445 Strip well format.

www.lsbio.com

Reagents for up to 96 tests.

Rev: February 2014

INTENDED USE

This mouse plasminogen total assay is for the quantitative determination of total plasminogen and plasmin in mouse plasma, serum, urine, cell culture media, or tissue extracts. **For research use only.**

BACKGROUND

Plasminogen is a single chain glycoprotein zymogen and is the precursor of the fibrinolytic enzyme plasmin. Plasminogen deficiencies are classified as hypoplasminogenemia (Type 1) or dysplasminogenemia (Type 2) and are associated with decreased extracellular fibrin clearance leading to mucous membrane lesions and ligneous conjunctivitis [1].

ASSAY PRINCIPLE

Mouse plasminogen will bind to the capture antibody coated on the microtiter plate. Plasminogen, plasmin, and plasmin in complex with antiplasmin will react with the antibody on the plate. After appropriate washing steps, polyclonal anti-mouse plasminogen primary antibody binds to the plasminogen. Excess antibody is washed away and bound polyclonal antibody is then reacted with the secondary antibody conjugated to horseradish peroxidase. Following an additional washing step, TMB substrate is used for color development at 450nm. The amount of color development is directly proportional to the concentration of total plasminogen in the sample.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-mouse plasminogen antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Mouse plasminogen standard:** 1 vial lyophilized standard
- **Anti-mouse plasminogen 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 standard 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 H₂SO₄ 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, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. 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 500ng/ml standard solution.

Dilution table for preparation of mouse plasminogen standard:

Plasminogen concentration (ng/ml)	Dilutions
500	Directly from vial
250	500 μl (BB) + 500 μl (500ng/ml)
100	600 μl (BB) + 400 μl (250ng/ml)
50	500 μl (BB) + 500 μl (100ng/ml)
20	600 μl (BB) + 400 μl (50ng/ml)
10	500 μl (BB) + 500 μl (20ng/ml)
5	500 μl (BB) + 500 μl (10ng/ml)
2.5	500 μl (BB) + 500 μl (5ng/ml)
1	600 μl (BB) + 400 μl (2.5ng/ml)
0	500 μl (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 100 μl plasminogen 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 300 μl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures plasminogen and plasmin antigens in the 1-500 ng/ml range. If the unknown is thought to have high plasminogen/plasmin levels, dilutions may be made in a similar biological fluid devoid of plasminogen or in blocking buffer. A 1:10,000 dilution for normal mouse plasma is suggested for best results.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100 μl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Secondary Antibody Addition

Briefly centrifuge vial before opening. Dilute 2 μl of conjugated secondary antibody in 10ml of blocking buffer and add 100 μl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300 μl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100 μl TMB substrate to all wells and shake plate for 1-5 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 μl 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.

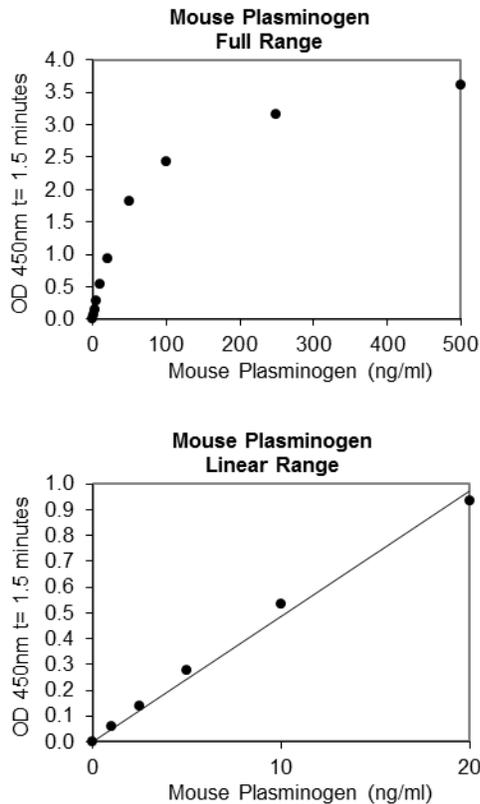
Measurement

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 A₄₅₀ against the amount of plasminogen 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 plasminogen 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 of human plasminogen in pooled donor plasma from normal individuals was found to be $195 \pm 10 \mu\text{g/ml}$ [2]. Normal values of plasminogen in mouse plasma have not been conclusively determined but are believed to be similar to human plasma. Plasminogen antigen was found to be $84 \pm 8 \mu\text{g/ml}$ in a small sample ($n=4$) of normal mice [3].

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 OD₄₅₀: 0.107-0.116) and calculating the corresponding concentration. The MDD was 0.28 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)	8.40	13.2	150
Standard Deviation	0.575	0.884	11.4
CV (%)	6.85	6.72	7.61

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)	7.93	14.0	149
Standard Deviation	0.736	1.33	8.76
CV (%)	9.29	9.51	5.87

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)	6.85	24.1	71.2	175
Average % Recovery	114	96	99	88
Range	110-123%	95-99%	92-99%	87-91%

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

Sample n	1:2	1:4	1:8	1:16
	4	4	4	4
Average % of Expected	99	98	103	101
Range	97-103%	94-106%	99-103%	94-105%

Specificity: This assay recognizes natural mouse plasminogen, plasmin, and plasmin/antiplasmin complex.

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

Sample Type	Dilution	Mean (µg/mL)
Citrate Plasma	1:10,000	223
	1:100,000	231

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	1 ng/ml	2.5 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	250 ng/ml	500 ng/ml		
B	0	1 ng/ml	2.5 ng/ml	5 ng/ml	10 ng/ml	20 ng/ml	50 ng/ml	100 ng/ml	250 ng/ml	500 ng/ml		
C												
D												
E												
F												
G												
H												

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. Tefs K, *et al.*: Blood. 2006, 108(9):3021-26.
2. Zolton RP, *et al.*: Clin Chem. 1972, 18:654-7.
3. Ploplis VA, *et al.*: Circulation. 1995, 92:2585-93.

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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