

LSBio™ Mouse Fibrinogen ELISA Kit

Catalog No. LS-F10440

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.

INTENDED USE

This mouse fibrinogen antigen assay is intended for the quantitative determination of total fibrinogen antigen in mouse plasma and serum. **For research use only.**

BACKGROUND

Fibrinogen is a soluble glycoprotein that circulates in the blood and is converted to insoluble fibrin by thrombin in the final step of the coagulation cascade [1]. Hepatic expression of fibrinogen increases two to four hundred fold during the acute phase response to infection or inflammation [2]. Elevated fibrinogen levels are correlated with cardiovascular disease [3] and atherosclerosis [4].

ASSAY PRINCIPLE

Mouse fibrinogen will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, biotin labeled polyclonal anti-mouse fibrinogen primary antibody binds to the captured protein. Excess antibody is washed away and bound polyclonal antibody is reacted with avidin 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 mouse fibrinogen. Color development is proportional to the concentration of fibrinogen in the samples.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-mouse fibrinogen antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **5X Diluent:** 1 bottle of 50ml
- **Mouse fibrinogen standard:** 1 vial lyophilized standard
- **Anti-mouse fibrinogen primary antibody:** 1 vial lyophilized polyclonal antibody
- **Horseradish peroxidase-conjugated Avidin:** 1 vial concentrated HRP labeled avidin
- **TMB substrate solution:** 1 bottle of 10ml solution
- **Stop solution:** 1 bottle of 6ml 1N H₂SO₄

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

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

- **1X Diluent:** 5X Diluent may contain precipitate. Warm to redissolve before use. Dilute 50ml of 5X diluent concentrate with 200ml of deionized water.
- **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 $\leq -20^{\circ}\text{C}$. 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 5ml of diluent directly to the vial and agitate gently to completely dissolve contents. This will result in a 800ng/ml standard solution.

Dilution table for preparation of mouse fibrinogen

standard:

Fibrinogen concentration (ng/ml)	Dilutions
800	Straight from the vial
400	500 μl Diluent + 500 μl (800ng/ml)
200	500 μl Diluent + 500 μl (400ng/ml)
100	500 μl Diluent + 500 μl (200ng/ml)
50	500 μl Diluent + 500 μl (100ng/ml)
25	500 μl Diluent + 500 μl (50ng/ml)
12.5	500 μl Diluent + 500 μl (25ng/ml)
6.25	500 μl Diluent + 500 μl (12.5ng/ml)
3.125	500 μl Diluent + 500 μl (6.25ng/ml)
0	500 μl Diluent 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 fibrinogen 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 total mouse fibrinogen in the 3.125-800 ng/ml range. If the unknown is thought to have high fibrinogen levels, dilutions may be made in diluent. It is suggested that normal mouse plasma samples be diluted between 1:50,000 and 1:100,000 in diluent for best results.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of diluent 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.

Avidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 2 μl of conjugated avidin in 10ml of diluent to generate a 1:5,000 dilution. Add 100 μl of the 1:5,000 dilution 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 5-15 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 μl 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 by gently shaking the plate.

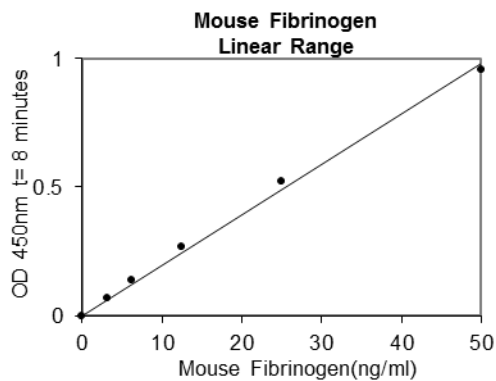
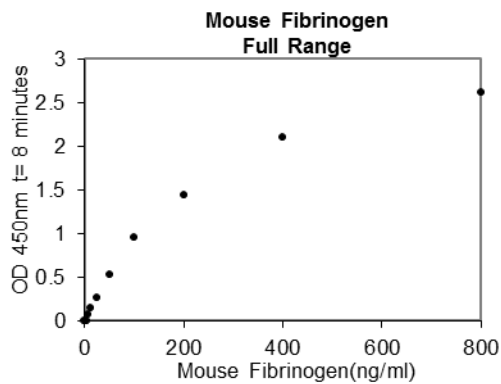
Measurement

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 fibrinogen 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 fibrinogen 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 fibrinogen in normal mouse plasma ranges from 1.4 to 2.1 mg/ml and varies by strain and

diet [5].

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: 0.064 -0.079) and calculating the corresponding concentration. The MDD was 0.289 ng/ml.

Intra-assay Precision: These studies are currently in progress. Please contact us for more information.

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

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

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. Kamath S and Lip GYH: QJM. 2003, 96:711-729.
2. Kusher I: Ann New York Acad Sci. 1982, 389:39-48.
3. Kannel WB, *et al.*: J Am Med Assoc. 1987, 258:1183-1186.
4. Hanga K, *et al.*: Atherosclerosis. 1989, 77:209-213.
5. Rezaee F, *et al.*: Atherosclerosis. 2002, 164:37-44.

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	3.125 ng/ml	6.25 ng/ml	12.5 ng/ml	25 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	400 ng/ml	800 ng/ml		
B	0	3.125 ng/ml	6.25 ng/ml	12.5 ng/ml	25 ng/ml	50 ng/ml	100 ng/ml	200 ng/ml	400 ng/ml	800 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, Cancellations: 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 cancellations 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.



2401 Fourth Avenue Suite 900 Seattle, WA 98121

Tel: 206.374.1102

Fax: 206.577.4565

Technical.Support@LSBio.com