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Mouse Fibrinogen ELISA Kit

Catalog No: CSI20057A
CSI20057B

Size: 1 x 96 tests
5 x 96 tests

Sensitivity:	0.059 ng/ml
Specificity:	Mouse fibrinogen
Range:	3-800 ng/ml
Sample Type:	Mouse serum and plasma

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. Hepatic expression of fibrinogen increases two to four hundred-fold during the acute phase response to infection or inflammation. Elevated fibrinogen levels are correlated with cardiovascular disease and atherosclerosis.

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:

Description	Quantity
CSI20057A – P. 96-well microtiter strip plate coated with anti-Mouse fibrinogen antibody, blocked and dried.	1 plate: 96 wells (12 strips x 8 wells)
CSI20057A - A. Wash Buffer Concentrate (10x)	1 bottle, 50 mL
CSI20057A – B. Diluent Concentrate (5X)	1 bottle, 50 mL
CSI20057A - C. Mouse fibrinogen standard, lyophilized	1 vial
CSI20057A - D. Anti-Mouse fibrinogen primary antibody, lyophilized polyclonal	1 vial
CSI20057A – E. Horseradish peroxidase-conjugated Avidin	1 vial
CSI20057A – F. TMB substrate solution	1 bottle, 10 ml
CSI20057A – G. Stop Solution, 1N H_2SO_4	1 bottle, 6 ml

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Storage and Stability:

All kit components must be stored at 2-8°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -80°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 2-8°C. Kit should be used no later than the expiration date.

Reagents and Equipment Required:

- Pipettes covering 0-10 µl and 200-1000 µl, and tips
- 12-channel pipette covering 30-300µl
- Paper towels or laboratory wipes
- Polypropylene conical 50 ml tubes, 1.5 ml flip-cap tubes
- Deionized or distilled water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450 nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm
- Automatic plate washer or wash bottle

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:

- **1X Diluent:** Dilute 50 ml of 5X Diluent Concentrate (CSI20057A-B) with 200 ml deionized water
- **1X Wash buffer concentrate:** Dilute 50 ml of 10X wash buffer with 450 ml deionized water

Specimen Collection:

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or aliquot and store at ≤ - 20°C. Avoid repeated freeze-thaw cycles.

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Assay Procedure: Allow microtiter strips and assay components to warm to room temperature for 30 minutes. Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard:
Reconstitute Standard by adding 5**.0 ml of Diluent** directly to the vial and agitate gently to completely dissolve contents. This will result in an 800 ng/ml standard solution.

Table 1: Dilution table for preparation of Human IgM standard:

Fibrinogen Concentration (ng/ml)	Dilutions
800	Straight from the vial
400	500 µl (BB) + 500 µl (800ng/ml)
200	500 µl (BB) + 500 µl (400 ng/ml)
100	500 µl (BB) + 500 µl (200 ng/ml)
50	500 µl (BB) + 500 µl (100 ng/ml)
25	500 µl (BB) + 500 µl (50 ng/ml)
12.5	500 µl (BB) + 500 µl (25 ng/ml)
6.25	500 µl (BB) + 500 µl (12.5 ng/ml)
3.125	500 µl (BB) + 500 µl (6.25 ng/ml)
0	500 µl (BB) Zero point to determine background

NOTE: Dilutions for the standard curve must be made and applied to the plate immediately.

Standard and Unknown Addition:
Remove microtiter plate from bag. Add 100 µl of fibrinogen standards in duplicate and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipes.

NOTE: The assay measures total mouse fibrinogen antigen in the 3.125 - 800 ng/ml range. If the unknown is thought to have higher fibrinogen levels, dilutions may be made in blocking buffer. A 1:50,000 to 1:100,000 dilution for normal mouse serum or plasma with diluent is suggested for best results.

Primary Antibody Addition:
Briefly centrifuge vial before opening. Reconstitute primary antibody by adding 10 ml of diluent directly to the vial and agitate gently to completely dissolve contents. Add 100 µl to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or laboratory wipe.

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Avidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 2.5µl of HRP conjugated avidin into 2.5ml diluent to generate a 1:1,000 dilution. Add 0.1ml of 1:1,000 dilution to 9.9ml of diluent to generate a 1:100,000 dilution. Add 100µl of the 1:100,000 dilution 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 100 µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different intensities 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 and read plate immediately.

Measurement:

Set the absorbance at 450 nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450 nm. 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.

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												

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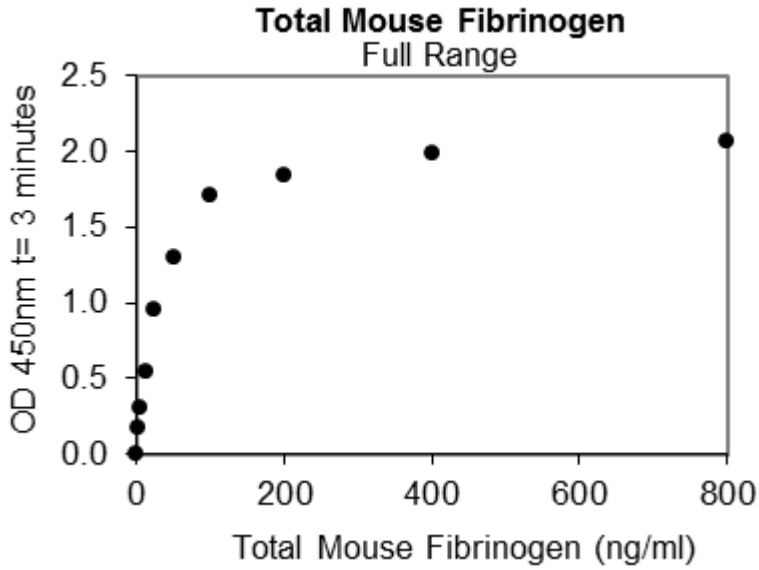
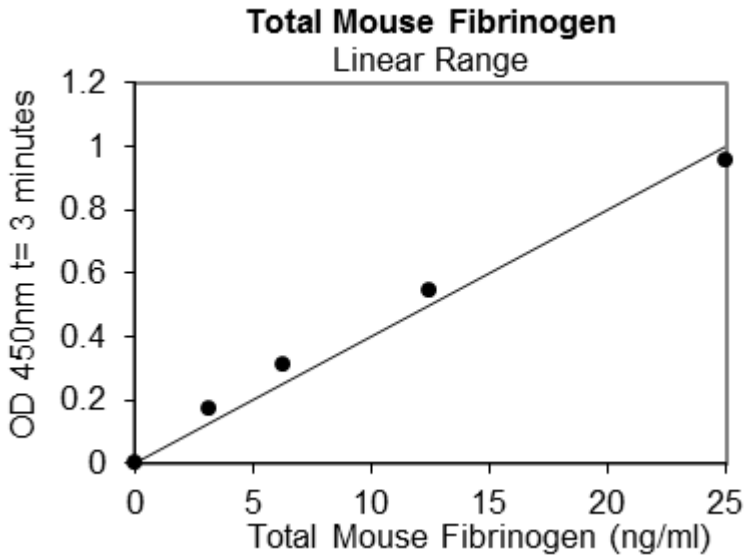
A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





Expected Value: The concentration of fibrinogen in normal mouse plasma ranges from 1.4 – 2.1 mg/ml and varies by strain and diet.

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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}: 0.056-0.061) and calculating the corresponding concentration. The MDD was 0.059 ng/ml.

Specificity: 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.

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.

Disclaimer: This information is believed to be correct but does not claim to be all-inclusive and should 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.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE

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