Mouse Fibrinogen Antigen Assay

Catalog No. IMFBGNKT Lot No. 311

Intended Use

This mouse fibrinogen antigen assay is intended for the quantitative determination of total fibrinogen antigen in mouse plasma and serum.

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 microtiter strip plate (8X12 removable wells):
Fibrinogen capture antibody coated

5X Diluent:
1 bottle of 50ml; bring to 1X using DI water

10X Wash Buffer:
1 bottle of 50ml; bring to 1X using DI water

Mouse fibrinogen antigen standard:
1 vial of lyophilized standard

Anti-mouse fibrinogen primary antibody:
1 vial of lyophilized biotin labeled polyclonal antibody

Avidin peroxidase secondary reagent:
1 vial of concentrated HRP labeled avidin

TMB substrate solution:
1 bottle of 10ml solution

Stop solution:
1 bottle of 6ml 1M sulfuric acid

Storage and Stability

All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°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 4°C. Kit should be used no later than the expiration date.

Required Reagents and Equipment

1-channel pipettes covering 0-10µL and 200-1000µL
12-channel pipette covering 30-300µL
Paper towels or kimwipes
50mL tubes, 1.5mL centrifuge tubes
1N H_2SO_4
DI water
Magnetic stirrer and stir-bars
Plastic containers with lids
Microtiter plate spectrophotometer operable at 450nm
Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

Warnings and Precautions

Warning – Avoid skin and eye contact when using TMB substrate solution. It may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

- 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.

Preperation of Reagents

Diluent concentrate: The diluent is supplied in a 5X concentrate and must be diluted 1:5 with deionized water for use with the kit.
Wash buffer concentrate: The wash buffer is supplied in a 10X concentrate and must be diluted 1:10 with deionized water for use with the kit.

Specimen Collection

The assay measures total mouse fibrinogen in the 3.125-800 ng/ml range. Samples giving mouse fibrinogen levels above 800ng/ml should be diluted in 1X diluent before use. Normal plasma samples need to be diluted between 1:50,000 and 1:100,000 in 1X diluent for the values to be within linear range of the standard curve.

Assay Procedure

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay. If a microtiter plate shaker is not available then keep the plate on a flat surface for 60 minutes at each step instead of 30 minutes.
Note: when the assay is performed without shaking the plate, the final absorbance values at 450nm will be lower than when the assay is performed using a plate shaker.

Preparation of Standard:

Reconstitute standard vial with 5 ml of 1X diluent to give a 800ng/ml solution.

Dilution table for preparation of human fibrinogen standards:

Fibrinogen concentration (ng/mL)	Dilutions
800	*Straight from the vial*
400	*500µl (1X Diluent) + 500µl (800ng/ml)*
200	*500µl (1X Diluent) + 500µl (400ng/ml)*
100	*500µl (1X Diluent) + 500µl (200ng/ml)*
50	*500µl (1X Diluent) + 500µl (100ng/ml)*
25	*500µl (1X Diluent) + 500µl (50ng/ml)*
12.5	*500µl (1X Diluent) + 500µl (25ng/ml)*
6.25	*500µl (1X Diluent) + 500µl (12.5ng/ml)*
3.125	*500µl (1X Diluent) + 500µl (6.25ng/ml)*
0	*500µl (1X Diluent) Zero point to determine b/g*

Standard and Unknown Addition:

Remove microtiter plate from bag. Add 100ul 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 300ul wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Primary Antibody Addition:

Add 10ml of 1X diluent directly to the primary antibody vial and agitate gently to completely dissolve contents. Add 100ul to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300ul wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Assay Procedure Continued

Secondary Reagent Addition:

Dilute 2.5µl into 2.5ml of 1X diluent and mix well. Add 1ml of diluted secondary reagent to 9ml of 1X diluent and add 100ul to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300ul wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation:

Add 100ul of TMB substrate solution to all wells and shake plate at 300rpm for 5-15 minutes. Quench the reaction with the addition of 50ul of 1N H2SO4 and read final absorbance values at 450nm.
NOTE: Time for substrate development is dependent on needs of researcher.

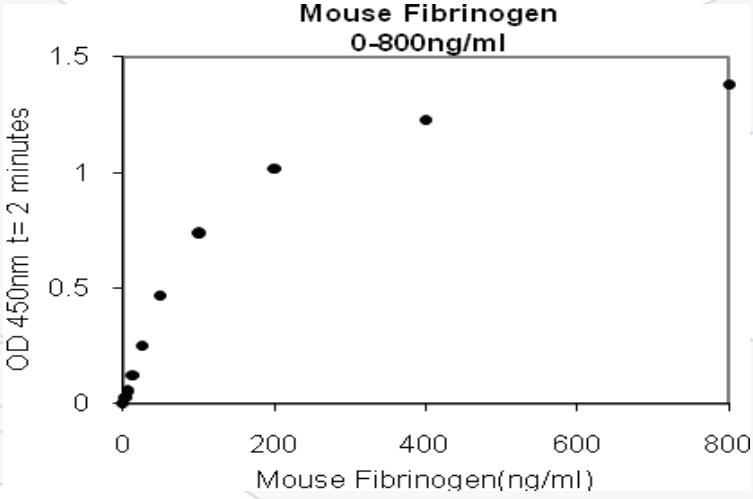
Measurement:

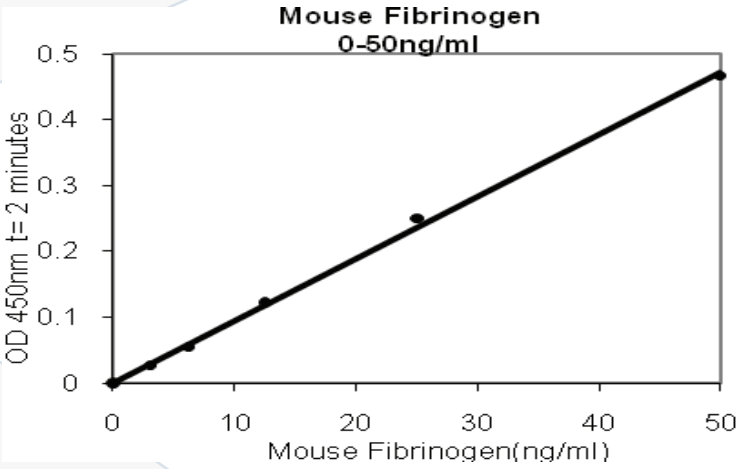
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm, A450. Subtract zero point from all standards and unknowns to determine corrected absorbance (A450).

Assay Calibration:

Plot A450 against the amount of human fibrinogen in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total human fibrinogen in the unknowns can be determined from this curve.

Standard Curve Examples





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].

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 or from contact with the above product.