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Human Fibrinogen ELISA Kit

Catalog No: CSI20049A
CSI20049B

Quantity: 1 kit, 1 x 96 tests/kit
5 kits, 1 x 96 tests/kit

INTENDED USE:

The human fibrinogen antigen assay is intended for the quantitative determination of total fibrinogen antigen in human 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. Hepatic 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:

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

REAGENTS PROVIDED:

Items	**Quantity Supplied with each Kit**
CSI20049-A. Microtiter strip plate coated with fibrinogen capture antibody	1 96-well plate (with 8 x 12-well removable strips)
CSI20049-B. Assay Diluent (5x)	1 bottle of 50 ml
CSI20049-C. Wash Buffer Concentrate (10x)	1 bottle of 50 ml
CSI20049-D. Human Fibrinogen Antigen Standard	1 vial (Lyophilized)
CSI20049-E. Anti-Human Fibrinogen Primary Antibody	1 vial (Lyophilized)
CSI20049-F. HRP-conjugated Streptavidin	1 vial concentrated HRP-streptavidin
CSI20049-G. TMB Substrate Solution*	1 bottle of 10 ml
CSI20049-H. Stop Solution	1 bottle of 6 ml 1 N H_2SO_4

***Hazard Information:** Avoid skin and eye contact when using TMB substrate solution as it may be irritating to eyes, skin and respiratory system. Wear safety goggles and gloves.

Cell Sciences ®
65 Parker Street
Unit 11
Newburyport, MA 01950

Toll Free: 888 769-1246
Phone: 978 572-1070
Fax: 978 992-0298

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

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 antibody 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.

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:

- **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 re-dissolve before use. Dilute 50 ml of 5X diluent concentrate with 200 ml of deionized water.
- **1X Wash buffer:** Dilute 50 ml of 10X wash buffer concentrate with 450 ml 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 (300 rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 2 ml of 1X Diluent directly to the vial and agitate gently to completely dissolve contents. This will result in an 800 ng/ml standard solution.

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Dilution table for preparation of Human Fibrinogen Standard:

Fibrinogen Concentration (ng/ml)	Dilutions
800	Straight from the vial
400	500 µl (1X Diluent) + 500 µl (800 ng/ml)
200	500 µl (1X Diluent) + 500 µl (400 ng/ml)
100	500 µl (1X Diluent) + 500 µl (200 ng/ml)
50	500 µl (1X Diluent) + 500 µl (100 ng/ml)
25	500 µl (1X Diluent) + 500 µl (50 ng/ml)
12.5	500 µl (1X Diluent) + 500 µl (25 ng/ml)
6.25	500 µl (1X Diluent) + 500 µl (12.5 ng/ml)
3.125	500 µl (1X Diluent) + 500 µl (6.25 ng/ml)
0	(500 µL Diluent) Zero point to determine background

NOTE: Dilutions for the standard curve and zero 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 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 kimwipe.

NOTE: The assay measures fibrinogen antigen 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 human plasma samples be diluted 1:100,000 to 1:1,000,000 in diluent, and normal human serum samples be diluted 1:10,000 to 1:50,000 in diluent.

Primary Antibody Addition

Reconstitute primary antibody by adding 10 ml of 1X 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 kimwipe.

Streptavidin-HRP Addition

Briefly centrifuge the vial before opening. Dilute 2.5 µl of HRP-conjugated streptavidin into 2.5 ml 1X Diluent to generate a 1:1,000 dilution. Add 0.1 ml of the 1:1,000 dilution to 9.9 ml of 1X Diluent to obtain a 1:100,000 dilution. Add 100 µl of the 1:100,000 dilution 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 kimwipe.

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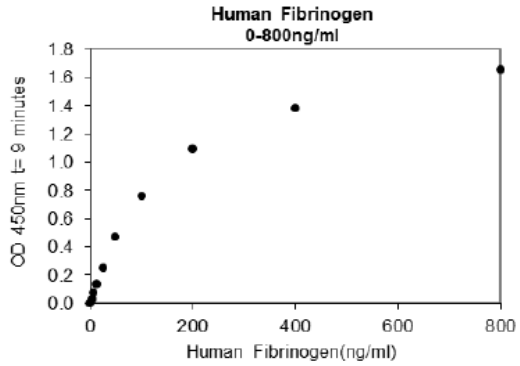
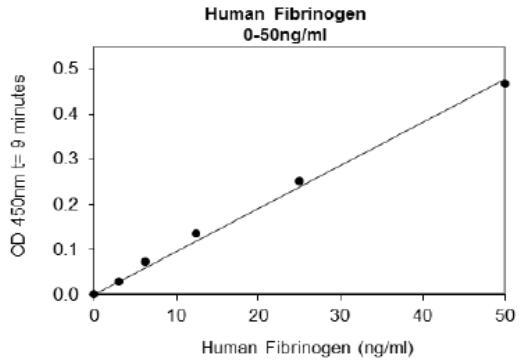
Substrate Incubation
Add 100 µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50 µl of 1N H_2SO_4 stop solution to all wells when samples are visually in the same range as the standards. Add 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 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.

A typical standard curve (EXAMPLE ONLY):





EXPECTED VALUES

The concentration of fibrinogen in normal human plasma ranges from 1.5 to 4.5 mg/ml. Elevated plasma fibrinogen levels are associated with a prothrombotic or hypercoagulative state and increased risk for ischemic heart disease and stroke.

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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}: 0.177-0.126) and calculating the corresponding concentration. The MDD was 0.41 ng/ml.

Specificity: This assay recognizes native human fibrinogen. Pooled normal plasma from mouse, rabbit, rat, horse, pig, dog, sheep, cynomolgus and rhesus monkey were assayed and no significant cross-reactivity were observed.

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												

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