

INTENDED USE

This human fibrinogen antigen assay is intended for the quantitative determination of total fibrinogen antigen in human 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

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

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

STANDARD CALIBRATION

Fibrinogen standard provided is calibrated against the WHO 3rd International Standard for Fibrinogen, Plasma, Human distributed by NIBSC (09/264), South Mimms, Potters Bar, Hertfordshire, UK.

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 2ml of diluent directly to the vial and agitate gently to completely dissolve contents. This will result in an 800ng/ml standard solution.

Dilution table for preparation of human 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 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 1:100 in diluent.

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.

Streptavidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 2.5 μl of HRP conjugated streptavidin into 2.5ml diluent to generate a 1:1,000 dilution. Add 0.2ml of 1:1,000 dilution to 9.8ml of diluent to generate a 1:50,000 dilution. Add 100 μl of the 1:50,000 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 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.

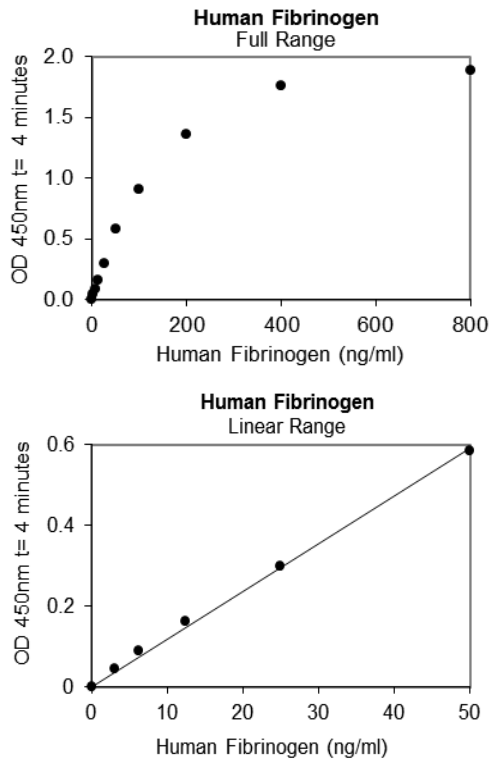
Measurement

Set the absorbance at 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 human plasma ranges from 1.5 to 4.5 mg/ml [5]. Elevated plasma fibrinogen levels are associated with a prothrombotic or hypercoagulative state and increased risk for ischaemic heart disease and stroke [1].

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} : 0.131-0.142) and calculating the corresponding concentration. The MDD was 0.22 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. Kuser 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. Lowe GDO, *et al.*: Ann Clin Biochem. 2004, 41:430-440.

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												

SAMPLE INSERT
Refer to kit box for
lot specific instructions