Linearity: To assess the linearity of the assay, human plasma samples containing normal concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

SAMPLE	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of expected	100	99	91	95
Range	98-102%	95-108%	89-95%	89-101%

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

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. 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. (ng/ml)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	3.125	6.25	12.5	25	50	100	200	400	800		
B	0	3.125	6.25	12.5	25	50	100	200	400	800		
C												
D												
E												
F												
G												
H												

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Human Fibrinogen ELISA Kit

Catalog # (SKU): IHUFBGKT

Strip well format.
Reagents for up to 96 tests.

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.

STANDARD CALIBRATION

The 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.

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 H_2SO_4

OTHER REAGENTS & 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 450nm
- 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.

STORAGE & STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standard 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.

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 ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE NOTES

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

SEE THE CERTIFICATE OF ANALYSIS (C of A) for SPECIFIC INSTRUCTIONS

Preparation of Standard: Reconstitute standard according to the C of A. Agitate gently to completely dissolve contents.

Dilution table for preparation of human fibrinogen standard:

Fibrinogen concentration (ng/ml)	Dilutions
800	Straight from the vial
400	500µl Diluent + 500µl (800ng/ml)
200	500µl Diluent + 500µl (400ng/ml)
100	500µl Diluent + 500µl (200ng/ml)
50	500µl Diluent + 500µl (100ng/ml)
25	500µl Diluent + 500µl (50ng/ml)
12.5	500µl Diluent + 500µl (25ng/ml)
6.25	500µl Diluent + 500µl (12.5ng/ml)
3.125	500µl Diluent + 500µl (6.25ng/ml)
0	500µl 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 & Unknown Addition: Remove microtiter plate from bag and add 100µl 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 300µl 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.

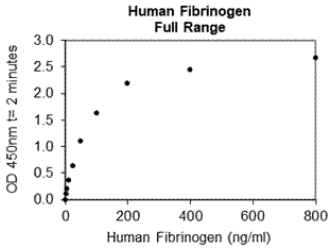
Primary Antibody Addition: Reconstitute primary antibody by adding 10ml of diluent directly to the vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping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

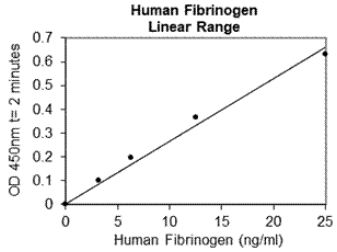
Secondary Antibody Addition Briefly centrifuge vial before opening. **See C of A for lot specific dilution instructions.** Add 100µl of your dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

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 ischemic heart disease and stroke [1].

Substrate Incubation: Add 100µl 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 50µl 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}).

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 and calculating the corresponding concentration. **See C of A for lot specifications.**

Intra-assay Precision: Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

SAMPLE	1	2	3
n	20	20	20
Mean (ng/ml)	1.03	5.06	411
Standard Deviation	0.027	0.456	39.6
CV (%)	2.62	9	9.62

Inter-assay Precision: Three samples of known concentration were tested in ten independent assays to assess inter-assay precision.

SAMPLE	1	2	3
n	10	10	10
Mean (ng/ml)	5.2	15.4	58.3
Standard Deviation	0.231	0.386	5.25
CV (%)	4.44	2.51	9.01

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in blocking buffer was evaluated.

SAMPLE	1	2	3	4
n	4	4	4	4
Mean (ng/mL)	0.26	4.95	34.09	168
Average % Recovery	102	99	95	110
Range	98-108%	95-104%	88-115%	105-116%