



AssayMax Human Fibrinogen (FBG) ELISA Kit (Plasma Samples)

Catalog No. EF1040-1

Introduction

Fibrinogen (FBG) is a homodimer of molecular mass 340 kDa, made up of two sets of α , β , γ polypeptide chains, and synthesized in the parenchymal cell of the hepatocyte and in the megakaryocyte (1). FBG plays a major role in coagulation, and both elevated and decreased levels have clinical significance. Upon cleavage by thrombin in the initial stages of coagulation activation, FBG self-assembles to yield a fibrin clot matrix that subsequently is crosslinked by factor XIIIa to form an insoluble network. FBG also binds to the platelet glycoprotein IIb/IIIa receptor so as to form bridges between platelets, thus facilitating aggregation (2). Elevated plasma FBG has been identified as an independent risk factor for coronary atherosclerosis and ischemic heart disease (3, 4). Individuals with congenital absence of FBG, termed afibrinogenemia, have prolonged bleeding times.

Principal of the Assay

The AssayMax Human Fibrinogen ELISA kit is designed for detection of human FBG in plasma. This assay employs a quantitative competitive enzyme immunoassay technique that measures FBG in less than 3 hours. A murine antibody specific for FBG has been pre-coated onto a 96-well microplate with removable strips. FBG in standards and samples is competed with a biotinylated FBG sandwiched by the immobilized antibody and streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-protein, and SP conjugate) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution

Reagents

- **FBG Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a murine antibody against FBG.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **FBG Standard:** Human FBG in a buffered protein base (480 µg, lyophilized).
- **Biotinylated FBG:** 1 vial, lyophilized.
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store components of the kit at 2-8⁰C or -20⁰C upon arrival up to the expiration date.
- Store SP Conjugate at -20⁰C
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8⁰C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8⁰C.
- Store Standard and Biotinylated Protein at 2-8⁰C before reconstituting with Diluent and at -20⁰C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000µl and multiple channel pipettes).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and use supernatants for assay. Dilute samples 1: 2000 into MIX Diluent. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA can also be used as anticoagulant).

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8⁰C.
- **Standard Curve:** Reconstitute the 480 µg of FBG Standard with 4 ml of MIX Diluent to generate a stock solution of 120 µg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (120 µg/ml) 1:3 with MIX Diluent to produce 40, 13.33, 4.44,

1.48, 0.49, and 0.16 µg/ml solutions. MIX Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20°C.

Standard Point	Dilution	[FBG] (µg/ml)
P1	1 part Standard (120 µg/ml) + 2 parts MIX Diluent	40.00
P2	1 part P1 + 2 parts MIX Diluent	13.33
P3	1 part P2 + 2 parts MIX Diluent	4.444
P4	1 part P3 + 2 parts MIX Diluent	1.481
P5	1 part P4 + 2 parts MIX Diluent	0.494
P6	1 part P5 + 2 parts MIX Diluent	0.165
P7	MIX Diluent	0.000

- **Biotinylated FBG (2x):** Dilute Biotinylated FBG with 4 ml MIX Diluent to produce a 2-fold stock solution. Allow the biotin to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution should be further diluted 1:2 with MIX diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

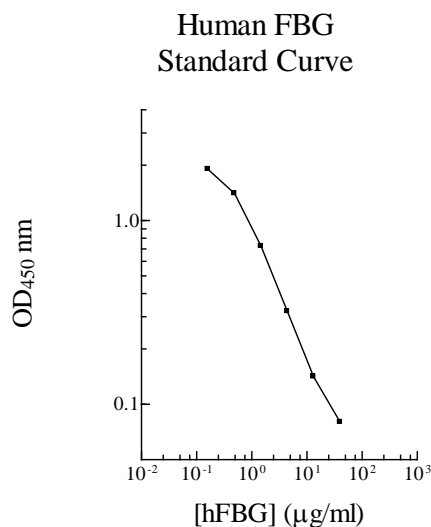
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25 µl of standard or sample per well and immediately add 25 µl of Biotinylated FBG to each well (on top of the Standard or sample). Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 8 minutes or till the optimal color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a Standard Curve, plot 4-parameter graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of FBG is typically ~0.16 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.1 % and 7.5% respectively.

Linearity

	Average Percentage of Expected Value
Sample Dilution	Plasma
1:1000	92%
1:2000	101%
1:4000	105%

Recovery

Standard Added Value	1 - 10 µg/ml
Recovery %	87-110 %
Average Recovery %	98.5 %

Reference Values

- Normal human plasma FBG concentration has been reported ranging approximately 2.26 to 3.3 mg/ml (5).

Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Bovine	None
Monkey	0.5%
Mouse	None
Rat	0.01%
Swine	0.5%
Rabbit	0.01%
Human	100%

References

- (1) Doolittle, R.F. (1984) *Annu. Rev. Biochem* 53:195
- (2) Handley, D.A. and Hughes, T.E. (1997) *Thromb. Res.* 87:1
- (3) Handa, K. *et al.* (1989) *Atherosclerosis* 77:209
- (4) Mannucci, P.M. and Mari, D. (1993) *Fibrinolysis* 3:51
- (5) Amiral J. (1995) *Clin. Appl. Thrombosis Hemostasis* 1:243

Version 7.4

Related Products

- EF2040-1 AssayMax Human Fibrinogen ELISA Kit (Urine, Milk, Saliva and Cell Culture Supernatant samples)
- ERF2040-1 AssayMax Rat Fibrinogen ELISA Kit (Urine and Cell Culture Supernatant samples)
- ERF1040-1 AssayMax Rat Fibrinogen ELISA Kit (Plasma samples)
- EMF2040-1 AssayMax Mouse Fibrinogen ELISA Kit (Urine and Cell Culture Supernatant samples)
- EMF1040-1 AssayMax Mouse Fibrinogen ELISA Kit (Plasma samples)