



AssayMax Human Fibrinogen (FBG) ELISA Kit (Urine and Cell Culture Supernatant Samples)

Catalog Number EF2040-1

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 cell culture media, tissue extract, and urine samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human FBG in 3.5 hours. A polyclonal antibody specific for human FBG has been pre-coated onto a 96-well microplate with removable strips. FBG in standards and samples is sandwiched by the immobilized polyclonal antibody and biotinylated polyclonal antibody specific for human FBG, which is recognized by a 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

- 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 polyclonal 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 (400 ng, lyophilized).

- **Biotinylated Human FBG Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against human FBG (80 µl).
- **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 (90 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 unopened kit at 2-8⁰C up to expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8⁰C. Store reconstituted reagents at -20⁰C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

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

Sample Collection, Preparation and Storage

- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20⁰C or below. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes and assay. Dilute samples 1:2 into MIX Diluent. Store samples at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8⁰C.
- **Standard Curve:** Reconstitute the 400 ng of FBG Standard with 5 ml of MIX Diluent to generate a standard solution of 80 ng/ml. Prepare triplicate standard points by serially diluting the standard solution (80 ng/ml) twofold with MIX Diluent to produce 40, 20, 10, 5, 2.5, and 1.25 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20⁰C.

Standard Point	Dilution	[FBG] (ng/ml)
P1	1 part Standanrd (80 ng/ml)	80.000
P2	1 part P1 + 1 part MIX Diluent	40.000
P3	1 part P2 + 1 part MIX Diluent	20.000
P4	1 part P3 + 1 part MIX Diluent	10.000
P5	1 part P4 + 1 part MIX Diluent	5.000
P6	1 part P5 + 1 part MIX Diluent	2.500
P7	1 part P6 + 1 part MIX Diluent	1.250
P8	MIX Diluent	0 .000

- **Biotinylated Human FBG Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20⁰C.
- **Wash Buffer Concentrate (20x):** 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 -20⁰C.

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 (20-30⁰C).
- 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 50 µl of standard or sample per well. 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. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Biotinylated Human FBG Antibody to each well and incubate for one hour.
- Wash five times with 200 µl of Wash Buffer.
- 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 five times with 200 µl of Wash Buffer.
- Add 50 µl of Chromogen Substrate per well and incubate for about 8 minutes or till the optimal blue 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**. Please note that after the reaction is stopped for about 10 minutes, some black particles may be generated at high concentration point, which will reduce the readings.

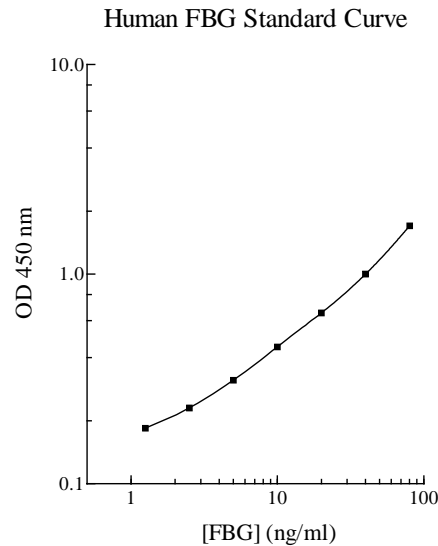
Data Analysis

- Calculate the mean value of the 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 500 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.5 % and 7.0% respectively.

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 3.3

Related Products

- EF1040-1 AssayMax Human Fibrinogen ELISA Kit (for plasma samples)