AssayMax Rat Fibrinogen (FBG) ELISA Kit

Catalog Number ERF1040-1
Lot #

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 IIbIIIa 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 Rat Fibrinogen ELISA kit is designed for detection of rat FBG in plasma. This assay employs a quantitative competitive sandwich 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 by 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

- 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:** Rat FBG in a buffered protein base (80 μg, lyophilized).
- **Biotinylated FBG (Biotin-FBG):** 1 vial, lyophilized.
- **Streptavidin-Peroxidase Conjugate (SP Conjugate)**: A 100-fold concentrate (120 μl).
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8^{0}C. Store reconstituted standard and Biotinylated FBG at -20^{0}C 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, 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 2,000x g for 10 minutes and use supernatants. Dilute samples 1: 800 into EIA Diluent and assay. The undiluted samples can be stored at -20^{0}C 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.

- **Standard Curve:** Reconstitute the 80 μg of FBG Standard with 1 ml of EIA Diluent to generate a stock solution of 80 μg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (80 μg/ml) 1:4 with EIA Diluent to produce 20, 5, 1.25, 0.313, and 0.078 μg/ml solutions. EIA Diluent serves as the zero standard (0 μg/ml).

Standard Point	Dilution	[FBG] (μg/ml)
P1	1 part Standanrd (80 μg/ml)	80.00
P2	1 part P1 + 3 part EIA Diluent	20.00
P3	1 part P2 + 3 part EIA Diluent	5.00
P4	1 part P3 + 3 part EIA Diluent	1.25
P5	1 part P4 + 3 part EIA Diluent	0.31
P6	1 part P5 + 3 part EIA Diluent	0.08
P7	EIA Diluent	0.00

- **Biotinylated FBG:** Dilute Biotinylated FBG with 4 ml EIA Diluent to produce a working solution. Any remaining solution should be frozen at < -20^0C.
- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent 1:10 with reagent grade water.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer Concentrate 1:10 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent.

Assay Procedure

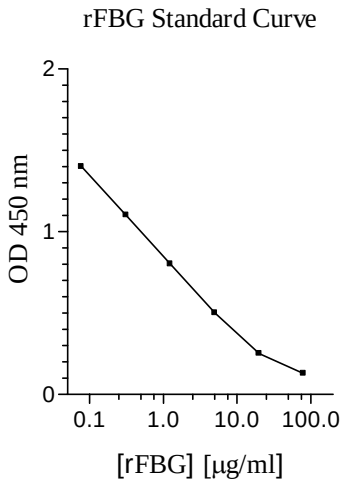
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^0C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal 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 2 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 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 as above.
- Add 50 μl of Chromogen Substrate per well and incubate for about 10 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**. 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 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 4-parameter or semi-log curve fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the plasma value by the dilution factor of 800.

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 20 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 6.8 % and 8.8% respectively.
- No cross react with human fibrinogen, 20% cross react with mouse Fibrinogen.

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

Revision 1.1

Related Products

- EF2040-1 AssayMax Human Fibrinogen ELISA Kit (for cell culture samples)
- EF1040-1 AssayMax Human Fibrinogen ELISA Kit (for plasma samples)