



# AssayMax Human Fibrinogen (FBG) ELISA Kit

Catalog Number EF1040-1

## Introduction

Fibrinogen (FBG) is a homodimer of molecular mass 340 kDa, made up of two sets of  $\alpha$ ,  $\beta$ ,  $\gamma$  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 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 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 (360 µg, lyophilized).
- **Biotinylated FBG (Biotin-FBG):** 1 vial, lyophilized.
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 µl).
- **MIX 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<sup>0</sup>C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8<sup>0</sup>C. Store reconstituted standard and Biotinylated FBG at -20<sup>0</sup>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 2000 x g for 10 minutes and use supernatants for assay. Dilute samples 1: 2000 into MIX Diluent as follows: add 20 µl of sample to 780 of MIX Diluent (1:40) to make Solution A; then add 20 µl of Solution A to 980 µl of MIX Diluent (1:50) to make a final working solution (1:2000). The undiluted samples can be stored at -20<sup>0</sup>C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.)

## 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 360 µg of FBG Standard with 3 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 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).

Standard Point	Dilution	[FBG] (µg/ml)
P1	1 part Standard (120 µg/ml)	120.00
P2	1 part P1 + 2 part MIX Diluent	40.00
P3	1 part P2 + 2 part MIX Diluent	13.33
P4	1 part P3 + 2 part MIX Diluent	4.44
P5	1 part P4 + 2 part MIX Diluent	1.48
P6	1 part P5 + 2 part MIX Diluent	0.49
P7	1 part P6 + 2 part MIX Diluent	0.16
P8	MIX Diluent	0.00

- **Biotinylated FBG:** Dilute Biotinylated FBG with 2 ml MIX Diluent to produce a 5-fold stock solution that can be further diluted 1:5 with MIX Diluent. Any remaining solution should be frozen at < -20°C.
- **MIX Diluent Concentrate (10x):** Dilute the MIX 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 MIX 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°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 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 completely 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 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**. Please note that some unstable black particles may be generated at high optical densities to reduce the readings after stopping the reaction for about 10 minutes.

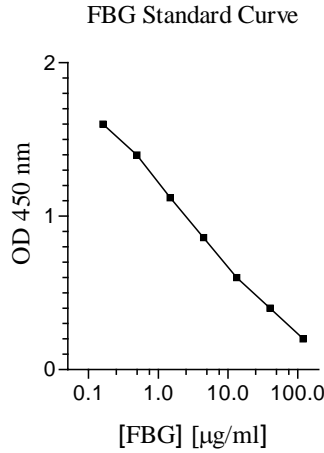
## Data Analysis

- 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 2000.

### Standard Curve

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



### Precision, Sensitivity and Specificity

- The minimum detectable dose of FBG is typically 100 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.0 % and 7.4% respectively.

### Recovery

<b>Standard Added Value</b>	0.5 - 40 µg/ml
<b>Recovery %</b>	87-110 %
<b>Average Recovery %</b>	98.5 %

### Linearity

<b>Sample Dilution</b>	Average Percentage of Expected Value
	Plasma
<b>1:1000</b>	102%
<b>1:2000</b>	100%
<b>1:4000</b>	105%

## Reference Values

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

## 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 6.1

## Related Products

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