



# Fibrinogen (Rat) ELISA Kit

Catalog Number KA0505

96 assays

Version: 02

Intended for research use only

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## **Introduction**

### **Background**

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.

### **Principle of the Assay**

The kit is designed for detection of rat FBG in cell culture media, and urine samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures rat FBG in 3.5 hours. A polyclonal antibody specific for rat 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 rat 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.

## General Information

### Materials Supplied

List of component

Component	Amount
FBG Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against FBG.	1
Sealing Tapes: pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.	3
FBG Standard™ Rat FBG in a buffered protein base	200 ng, lyophilized
Biotinylated Rat FBG Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against Rat FBG	140 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 , 2 bottles
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 hydroxychloric acid to stop the chromogen substrate reaction	12 ml

### Storage Instruction

- ✓ Store components of the kit at 2-8 °C or -20 °C upon arrival up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated Antibody at -20 °C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8 °C.
- ✓ 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 2-8 °C.
- ✓ Store Standard at 2-8 °C before reconstituting with Diluent and at -20 °C after reconstituting with Diluent.

### Materials Required but Not Supplied

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

### **Precautions for Use**

- Precautions
- ✓ Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-antibody, 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 and the biotinylated-antibody 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

## Assay Protocol

### 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 2-8°C.
- Standard Curve: Reconstitute the 200 ng of FBG Standard with 1 ml of MIX Diluent to generate a standard solution of 200 ng/ml. Prepare triplicate standard points by serially diluting the standard solution (200 ng/ml) 1:2 with MIX Diluent to produce 100, 50, 25, 12.5, 6.25 and 3.13 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C.

Standard Point	Dilution	[FBG] (ng/ml)
P1	1 part Standard (200ng/ml)	200.000
P2	1 part P1 + 1 part MIX Diluent	100.000
P3	1 part P2 + 1 part MIX Diluent	50.000
P4	1 part P3 + 1 part MIX Diluent	25.000
P5	1 part P4 + 1 part MIX Diluent	12.500
P6	1 part P5 + 1 part MIX Diluent	6.250
P7	1 part P6 + 1 part MIX Diluent	3.125
P8	MIX Diluent	0.000

- Biotinylated Rat FBG Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 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.

### Sample Preparation

- Cell Culture Supernatants: Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C 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 -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles

## **Assay Procedure**

1. 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).
2. 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.
3. 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.
4. 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.
5. Add 50 µl of Biotinylated Rat FBG Antibody to each well and incubate for one hour. Wash five times with 200 µl of Wash Buffer.
6. Wash the microplate as described above.
7. 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.
8. Wash the microplate as described above.
9. Add 50 µl of Chromogen Substrate per well and incubate for about 10 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.
10. Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
11. 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

### Calculation of Results

- ✓ 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.

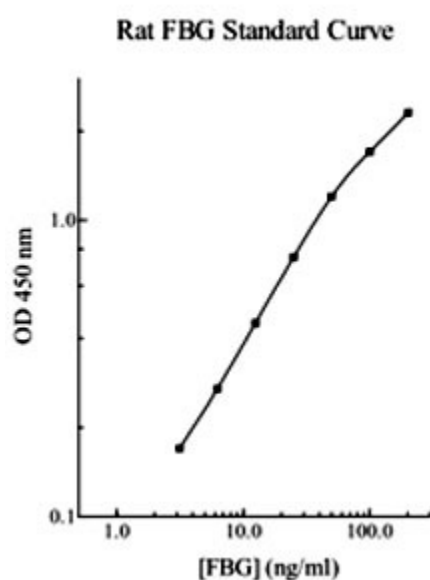


Figure 1: Typical Standard Curve for Fibrinogen (Rat) ELISA Kit

### Performance Characteristics

- ✓ The minimum detectable dose of FBG is typically 3 ng/ml.
- ✓ Intra-assay and inter-assay coefficients of variation were 5.1 % and 7.9% respectively.

- Linearity

	Average Percentage of Expected Value
Sample Dilution	Urine
No Dilution	89%
1:2	95%
1:4	91%

- Recovery

Standard Added Value	4 – 80 ng/ml
Recovery %	81-112 %
Average Recovery %	96 %

- Cross-Reactivity

Species	% Cross Reactivity
Canine	1%
Bovine	None
Monkey	5%
Rat	100%
Human	1%
Swine	None
Rabbit	None
Mouse	50%

## Resources

### References

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5. Amiral J. (1995) Clin. Appl. Thrombosis Hemostasis 1:243

**Plate Layout**

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