Fibrinogen ELISA Quatitation Kit (Human) (GWB-C5E724)

Instructions for use

For the quantitative measurement of fibrinogen in human serum, plasma, and other biological fluids.

Lot to lot variation can occur. Refer to the manual provided with the kit.

This product is intended for research use only.

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1. Background

Principle

GenWay Biotech Fibrinogen ELISA Kit (Human) (GWB-C5E724) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for fibrinogen is coated onto a 96-well plate. Standards or test samples are added to the wells, incubated and washed. A horseradish peroxidase (HRP) conjugated detector antibody specific for fibrinogen is added, incubated and followed by washing. An enzymatic reaction is produced through the addition of TMB substrate which is catalyzed by HRP generating a blue color product that changes yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm is quantitatively proportional to the amount of sample fibrinogen captured in well.

Target Background

Fibrinogen is a glycoprotein found in the blood of vertebrates. It is comprised of three pairs of nonidentical polypeptide chains. Following vascular injury, fibrinogen is cleaved by thrombin to form fibrin which is the most abundant component of blood clots. In addition, various cleavage products of fibrinogen and fibrin regulate cell adhesion and spreading, display vasoconstrictor and chemotactic activities, and are mitogens for several cell types. Mutations in the gene that encodes for fibrinogen lead to several disorders, including afibrinogenemia, dysfibrinogenemia, hypodysfibrinogenemia and thrombotic tendency. Alternatively, spliced transcript variants encoding different isoforms have been found for this gene.

General Specifications

General Specifications	
Range	6.25 ng/mL – 400 ng/mL
Limit of Detection (LOD)	0.313 ng/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Target Information	Human Fibrinogen UniProt ID: P02671 GeneID: 2243 Target Alias: Fibrinogen alpha chain; fibrinogen, A alpha polypeptide

2. Storage and Stability

Upon receipt, store kit at -20°C until the expiration date, which can be found on the Certificate of Analysis.

3. Kit Components

The following reagents are the provided contents of the kit:

Description	Quantity	Storage Conditions
Fibrinogen Capture Antibody	1 x 75 µL	Store at -20°C until the expiration date
Fibrinogen Standard	1 x 250 µL	
Fibrinogen HRP-Detection Antibody	1 x 70 µL	

4. Precautions

- Read instructions fully prior to beginning use of the assay kit.
- Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For information on hazardous substances included in the kit refer to the Material Safety Data Sheet (MSDS).
- Kit cannot be used beyond the expiration date on the label.

5. Required Materials Not Supplied

- 96-well High-Binding Microplate
- Microplate reader capable of reading absorbance at 450 nm
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Distilled or deionized ultrapure water.
- 1x PBS, pH 7.2~7.4
- Tween 20
- Nonfat dried milk.
- TMB Substrate
- Stop Solution (4 N H_2SO_4)
- 37°C Incubator (optional).

6. Technical Application Tips

- Do not mix or substitute components from other kits.
- To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
- Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
- Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.
- Replicate wells are recommended for standards and samples.
- Cover microplate while incubating to prevent evaporation.
- Do not allow the microplate wells dry at any point during the assay procedure.
- Do not reuse tips or tube to prevent cross contamination.
- Avoid causing bubbles or foaming when pipetting, or mixing.
- Completely remove of all liquids when washing to prevent cross contamination.
- Prepare reagents immediately prior to use and do not store, except for the top standard.
- For optimal results for inter- and intra-assay consistency, equilibrate all materials (except for standards) to 37°C prior to performing assay and perform all incubations at 37°C.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.
- Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
- Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.

- Samples that are hemolytic, lipemic, or contain precipitates, fibrin strands or bilirubin may cause inaccurate results due to interfering factors.
- TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

7. Reagent Preparation

- Equilibrate all materials to room temperature prior to use and prepare immediately prior to use.
- Prepare the **Fibrinogen Standard** no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.

7.1 Buffer Preparation

Prepare the following buffers:

7.1.1 Coating Buffer: 50 mM Carbonate-Bicarbonate, pH 9.6

7.1.2 Wash Solution: 0.05% Tween 20 in PBS, pH 7.4

7.1.3 Blocking Solution: 1% Nonfat Dry Milk Powder in PBS, pH 7.2~7.4

7.1.4 Sample Diluent: 1% Nonfat Dry Milk Powder in PBS, pH 7.2~7.4

7.1.5 Conjugate Diluent: 0.2% Nonfat Dry Milk Powder in PBS, pH 7.2~7.4 from Sample Diluent (Add 2.2 mL Sample Diluent into 8.8 mL PBS)

7.1.6 Enzyme Substrate: TMB

7.1.7 Stopping Solution: 4 N H_2SO_4 or other appropriate solution

7.2 Fibrinogen Coating Antibody

7.2.1 Prepare the **Fibrinogen Coating Antibody** by diluting 6.9 µL of **Fibrinogen Capture Antibody** solution in 11 mL of the **Coating Buffer**.

7.3 Standards and Samples

7.3.1 Dilute the Fibrinogen Standard to 400 ng/mL by adding 10 µL of the protein in 490 µL of **Sample Diluent**. This will be **Standard #1.**

7.3.2 Prepare a set of serially diluted standards as follows:

7.3.2.1 Label tubes with the respective standard number.

7.3.2.2 Add 250 µL of **Sample Diluent** into all the tubes

7.3.2.3 Prepare **Standard #2** by adding 250 µL of **Standard #1** from Tube #1 to Tube #2. Vortex briefly to mix.

7.3.2.4 Prepare **Standard #3** by adding 250 µL of **Standard #2** from Tube #2 to Tube #3. Vortex briefly to mix.

7.3.2.5 Prepare further serial dilutions in the remaining tubes.

7.3.2.6 The last tube is a blank standard (only **Sample Diluent**), which should be included with every experiment.

7.4 Fibrinogen HRP-Detection Antibody

7.4.1 Prepare the **Fibrinogen HRP-Detection Antibody** immediately prior to use by adding 5.5 ul of the **Fibrinogen HRP-Detection Antibody** to 11mL of **Conjugate Diluent**.

7.4.2 Mix thoroughly and gently. Do not store at 1x concentration for future use.

8. Sample Preparation

8.1 Sample Preparation and Storage

- Store samples to be assayed at 2-8°C for 24 hours prior to being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.
- This assay is intended for serum. The sample preparation protocols below have been provided for your reference:

    - **Serum** - Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

    - **Plasma** - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

    - **Tissue Homogenates** – Rinse 100 mg of tissue with 1X PBS, then homogenize in 1 mL of 1X PBS and stored overnight at -20°C. Perform two freeze-thaw cycles to break the cell membranes, then centrifuge the homogenates for 5 minutes at 5,000 x g, 2-8°C. Remove the supernatant and assay immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

    - **Cell culture supernatants** – Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

Note: Samples that are hemolytic, lipemic, or contain precipitates, fibrin strands or bilirubin may cause inaccurate results due to interfering factors

8.2 Sample Dilution

- Target protein concentration must be estimated, and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.
    - Dilute samples using **Sample Diluent**.
    - Mix diluted samples gently and thoroughly.
    - Pipetting less than 2 µL is not recommended for optimal assay accuracy.

9. Assay Procedure

Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

9.1 Add 100 µL **Fibrinogen Coating Antibody** into each well

9.2 Cover the plate with plate sealer and incubate for 60 minutes at 37° C or overnight at 4° C

9.3 Aspirate the **Fibrinogen Coating Antibody** solution from each well

9.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

9.5 Wash plate 3 times with **Wash Solution** as follows:

9.5.1 Add 300 µL of **Wash Solution** to each assay well.

9.5.2 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

9.5.3 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

9.5.4 Repeat steps 10.5.1 through 10.5.3 **two** more times.

9.5.5 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

9.6 Add 300 µL of **Blocking Solution** to each well

9.7 Cover the plate with a plate seal and incubate for 60 minutes at room temperature

9.8 After incubation, remove the **Blocking Solution** and wash each well three times as in step 9.5

9.9 Transfer 100 µL of the serially diluted **Fibrinogen Standard** solutions and sample solutions to designated wells

9.10 Cover the plate with a plate seal and incubate for 60 minutes at room temperature

9.11 After incubation, remove samples and standards and wash each well 5 times as in step 9.5

9.12 Transfer 100 µL of the freshly prepared **Fibrinogen HRP-Detection Antibody** solution to each well

9.13 Cover the plate with a plate seal and incubate for 60 minutes at room temperature

9.14 After incubation, remove the HRP conjugate and wash each well 5 times as in step 9.5

9.15 Add 100 µL of TMB Substrate to each well and incubate for 5-30 minutes in the dark**.** Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.

(NOTE: TMB Substrate must be pre-warmed to room temperature before adding to the plate. Incubation time must be determined by the user**.** Optimal development can be visualized by blue shading in the top four standard wells. The blank (final standard point) should be faint blue to clear.)

9.16 Add 50 µL of Stop Solution to each well. Well color should change to yellow immediately. Add the Stop Solution in the same well order as done for the TMB Substrate.

10. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

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11. References

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