Product Manual

Human D- dimer ELISA Kit

Catalog Numbers

PRB- 5048	96 assays
PRB- 5048- 5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Thrombosis is the local coagulation (clotting) of the blood in a part of the circulatory system. During blood vessel injury, fibrin and other clotting factors are produced to prevent excessive blood loss. Two key proteases are activated during this coagulation cascade which lead to fibrin formation, thrombin and Factor XIII. Thrombin converts soluble blood fibrinogen into insoluble fibrin mesh (proteofibrils); Factor XIII then crosslinks this fibrin mesh to form an insoluble gel which serves as a scaffold for blood clot formation (Figure 1). Conversely, during fibrinolysis process, the clot is degraded by plasmin to yield fibrin degradation products (FDPs). The most notable subtype of fibrin degradation products is the D-dimer fragment, which contains two D cross-linked domains and one E domain of the original fibrinogen molecule. Levels of D-dimer are typically low in human blood plasma, except when the coagulation system has been activated. D-dimer is a useful diagnostic biomarker for deep vein thrombosis, pulmonary embolism, disseminated intravascular coagulopathy, and treatment efficacy in acute myocardial infarction.

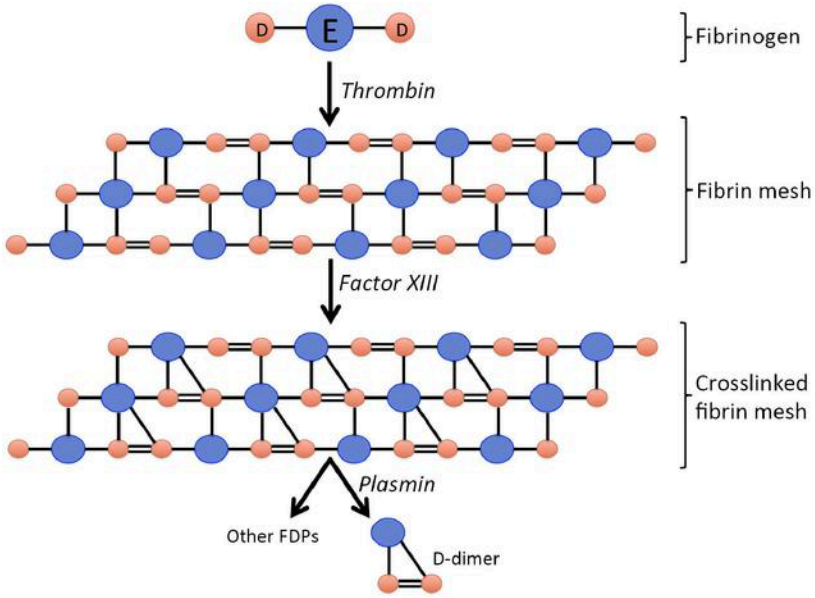

Figure 1: D-dimer Formation

Cell Biolabs' D-dimer ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the human D-dimer protein. The kit has detection sensitivity limit of 150 pg/mL D-dimer. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and D-dimer samples.

Note: This kit is D-dimer specific and does not react with fibrinogen or fibrinogen degradation products.

Assay Principle

An anti-D-dimer coating antibody is adsorbed onto a microtiter plate. D-dimer protein present in the sample or standard binds to the antibodies adsorbed on the plate; a biotinylated anti-D-dimer antibody is added and binds to the antigen captured by the first antibody. Following incubation and wash steps, a streptavidin-enzyme conjugate is added and binds to the biotinylated anti-D-dimer antibody. Unbound streptavidin-enzyme conjugate is removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of D-dimer present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from purified D-dimer and sample concentration is then determined.

Related Products

1. PRB-5033: Human Alpha 2 Macroglobulin ELISA Kit
2. PRB-5034: Human Alpha 1 Antitrypsin ELISA Kit
3. PRB-5038: Human Beta 2 Microglobulin ELISA Kit
4. PRB-5039: Human Haptoglobin ELISA Kit
5. PRB-5041: Human Ceruloplasmin ELISA Kit
6. PRB-5044: Human Alpha 1 Antitrypsin ELISA Kit
7. PRB-5047: Human CK-MB ELISA Kit
8. PRB-5050: Human Troponin I ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-D-dimer Antibody Coated Plate (Part No. 50481B): One strip well 96-well plate.
2. Biotinylated Anti-D-dimer Antibody (1000X) (Part No. 50482D): One 20 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human D-dimer Standard (Part No. 50483D): One 100 µL vial of 10 µg/mL human D-dimer.

Materials Not Supplied

1. D-dimer Sample: serum, plasma, lysate
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips

3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receiving, aliquot and store D-dimer Standard at -20ºC and avoid freeze/thaw. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-D-dimer Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-D-dimer Antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of D-dimer Standard in the concentration range of 10 ng/mL – 0.156 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	10 µg/mL Human D-dimer Standard (µL)	Assay Diluent (µL)	D-dimer (ng/mL)
1	4	3996	10
2	500 of Tube #1	500	5
3	500 of Tube #2	500	2.5
4	500 of Tube #3	500	1.25
5	500 of Tube #4	500	0.625
6	500 of Tube #5	500	0.313
7	500 of Tube #6	500	0.156
8	0	500	0

Table 1. Preparation of D-dimer Standard

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Add 100 µL of D-dimer sample or standard to the Anti-D-dimer Antibody Coated Plate. Each D-dimer sample, standard, blank, and control should be assayed in duplicate.
3. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.

4. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Biotinylated Anti-D-dimer Antibody to each well.
6. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
7. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 4 above.
8. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well.
9. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
10. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 4 above. Proceed immediately to the next step.
11. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
12. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
13. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical D-dimer ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

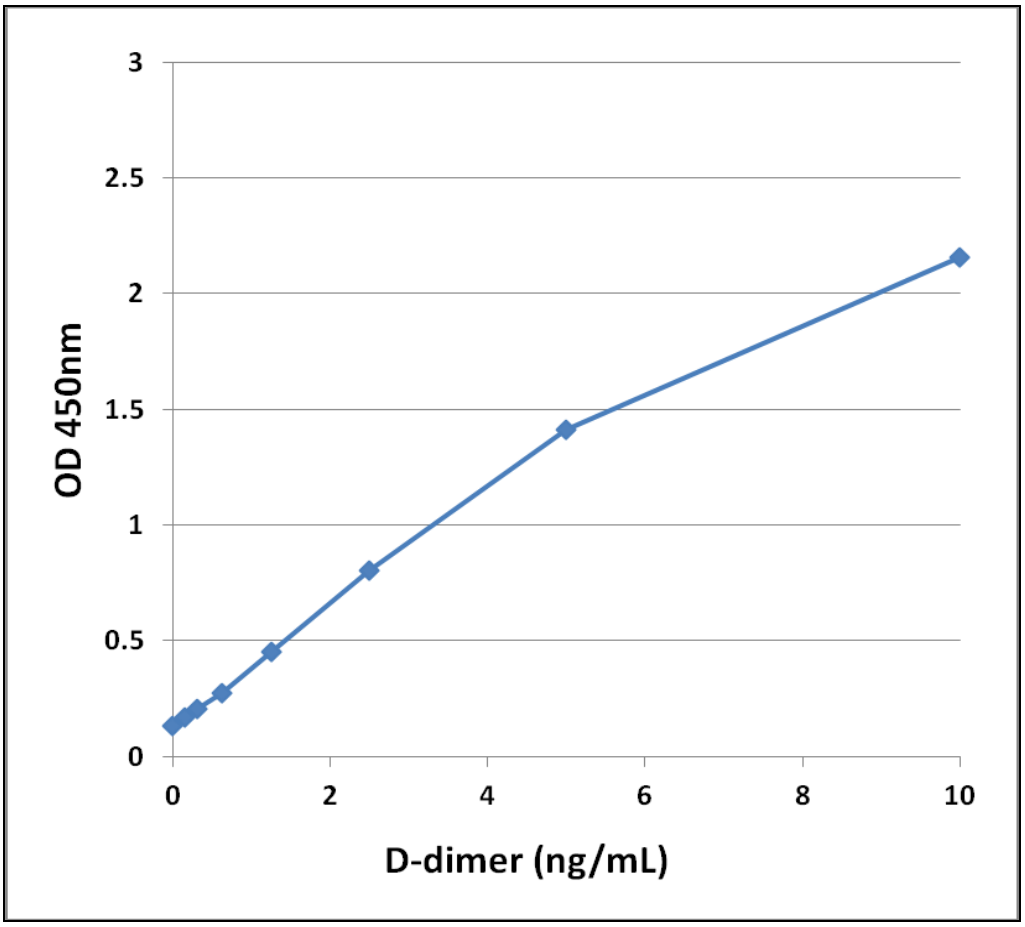

Figure 2: D-dimer ELISA Standard Curve

References

1. Anderson, D., P. Wells (2000) *Curr. Opin. Hematol.* **7**:296-301.
2. Cesarman-Maus, G., K. Hajjar (2005) *Br. J. Haematol.* **129**:307-321.
3. Mammen, E (2000) *Clin. Lab. Sci.* **13**:239-245.

Warranty

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