Plasma fibronectin is a high molecular weight glycoprotein composed of two nearly identical polypeptide chains (each 220 kD). It is synthesised and secreted by the liver and circulates at a concentration of approximately 330µg/mL.

Fibronectin belongs to the group of "Cell Attachment Proteins". Its dimeric structure allows it to function as a molecular glue, holding various molecules together through its binding domains. Principal binding domains exist for fibrin, heparin, Staphylococcus aureus, collagen and the cell surface. Fibronectin has been shown to play a role in fibrin clot formation, platelet function, fibrinolysis, chemotaxis, phagocytosis and opsonisation. Lowered plasma fibronectin levels are associated with sepsis, trauma and are also observed during the post-operative period.

Hepatic disorders are also often associated with reduced fibronectin levels. Fibronectin binds to fibrin through factor XIII and levels may be reduced by activation of the clotting system.

Fibronectin is an "Acute Phase Protein" and its level may be elevated during the acute phase. Certain malignancies are associated with high fibronectin levels and this protein may play a role in cell metastasis.

Application

The TC Fibronectin Test is used to measure human plasma fibronectin levels, particularly in patients with severe trauma, shock, sepsis, hepatic disease or clotting disorders.

Test principle

The TC Fibronectin Test is a solid phase enzyme immunoassay.

Specificity

The peroxidase conjugated anti-fibronectin monoclonal antibody used in this kit recognizes only intact fibronectin, i.e. split products of the molecule are not detected. In normal plasma, approximately two - thirds of the total quantity of circulating fibronectin is in intact form. In patients with severe septicaemia or disseminated intravascular coagulation (DIC) fibronectin split products are generated by the action of leucocyte elastase or plasmin on the intact molecule leading to a rapid decrease in the levels of functionally intact fibronectin. The normal plasma concentration of total fibronectin is 330 ± 80 µg/mL, and native uncleaved Fibronectin is 70 – 148 µg/mL.

Test samples

Use citrated plasma samples.

Collect the blood of patients into precooled plastic or siliconized tubes already containing 3.2% buffered citrate in a ratio of 1:10 (blood anticoagulant). Centrifuge within 30 minutes after the puncture at 2000g for 30min. at 4°C. Pipette off the plasma. Store as aliquots at temperature below -30°C.

Repetitive freeze/thawing of the plasma is not recommended.

Kit components

Determinations: Five 96 well microtitre plates (=210 samples in duplicate)

1. COATING ANTIBODY

1x lyophilized monoclonal anti Fibronectin coating antibody (TC-Code HK).

2. PEROXIDASE CONJUGATED ANTIBODY

5x peroxidase conjugated monoclonal anti-Fibronectin antibody (concentrated) (TC-Code AG).

3. STANDARD

5x lyophilized normal pooled human plasma, calibrated for Fibronectin (TC-Code AJ).

Kit storage: Store all components at 2-8°C

BUFFERS NEEDED

Coating buffer

1.59gNa2CO3, 10H2O
2.93gNaHCO3
0.01% Thimerosal
Distilled H2O to end volume 1 liter
pH 9.6

Phosphate buffered saline (PBS)

8.0 g NaCl
0.2 g KCl
0.2 g KH2PO4
1.44 g Na2HPO4.2H2O
Distilled H2O to end volume 1 liter
pH 7.4

Washing buffer

0.5% Tween 20 in PBS

Dilution buffer

1% bovine serum albumin (BSA) in PBS.

TMB substrate buffer

0.1M sodium-acetate
0.1M citric acid, pH=6

TMB substrate solution

add 10 mg TMB dihydrochloride per 100mL substrate buffer and mix to dissolve. Then add 20 µL 30% H2O2 solution.

Or use a commercially available ready to use TMB substrate solution suitable for use in ELISA systems.

The peroxidase labelled antibody has been optimised for specific use with TMB substrate. Use of other substrates is not recommended.

Stop solution

2M sulphuric acid

TWEEN 20=Polyoxyethylene sorbitan monolaurate.

Also required

Flat bottom 96-well ELISA plates such as: NUNC - Immuno Plate Maxisorb COSTAR - Serocluster EIA plate flat botttom FALCON - Pro-Bind Assay Plates GREINER - Flat Bottom ELISA Plates high binding capacity

Preparations

Amounts given are for one 96-well plate.

1. COATING THE PLATE

a) Restore the vial of coating antibody with 0.5 mL distilled water.

b) For one 96 well plate:

0.1 mL coating antibody + 10 mL coating buffer.

c) Pipette 0.1 mL of the diluted antibody solution into each well and cover the plate with a plastic foil.

d) Incubate the plate for at least 16 hours at 4°C.

Plate is stable at 4°C for 2 months (when sealed tightly).

e) Empty the antibody coated plate (discard the antibody solution) and tap dry on absorbant paper towel.

2. STANDARD CURVE

Prepare a standard curve from 0 to 0.025 µg/mL fibronectin.

3. SAMPLE DILUTIONS

Dilute the plasma samples 1:200 and 1:400 with dilution buffer.

4. POX-CONJUGATED ANTIBODY

Prepare the conjugate working solution (1+50): Dilute 1 part by volume conjugate with 50 parts by volume incubation buffer.

For 8 test wells: Mix 20 µl conjugate with 1000 µl incubation buffer.
Assay Procedure

1. **PLATE**
   Empty wells of buffer. Wash the plate three times with wash buffer by adding 0.25 mL wash buffer per well/cycles. Tap strips dry on absorbent paper.

2. **SAMPLE/STANDARD ADDITION**
   Pipette 0.1 mL of the diluted samples/standard into separate wells. Blank well is filled with 0.1 mL dilution buffer. Running standard/sample in duplicate is recommended.

3. **SAMPLE INCUBATION**
   Cover the plate with a plastic foil and incubate for 1 hour at 37°C.

4. **WASH PLATE**
   Wash three times as described in step 1.

5. **POX ANTIBODY ADDITION**
   Add 0.1 mL of the diluted POX anti-Fibronectin antibody to all wells, preferably with a multichannel pipette.

6. **POX ANTIBODY INCUBATION**
   Cover and incubate the plate for 30 minutes at 37°C.

7. **WASH PLATE**
   Wash three times as described in step 1.

8. **SUBSTRATE**
   Pipette 0.1 mL of TMB substrate to all wells. Incubate for 15 minutes at room temperature.

9. **STOP**
   Pipette 0.1 mL of stop solution to all wells.

10. **READ**
    Measure absorbances at 450 nm (with 620 nm reference filter if available). Read absorbances within one hour after the addition of the stop solution.

11. **GRAPH**
    Construct a graph of standard curve.

12. **Locate the absorbance for each sample on the curve and read the corresponding value from the horizontal axis. Do not forget to multiply by the dilution factor (200, 400 or 800) for the samples.**

   ![Typical standard curve (LogLin scale)](image)

### Evaluation of results

Only intact fibronectin is detected using this kit, which is approximately two-thirds of the total fibronectin, with a range of concentrations from 70 – 148 µg/mL.

### Notes

- Be sure to prepare all reagents before proceeding with the assay. It is critical to keep the time necessary for pipetting standards and samples to a minimum and avoid delays. Be sure to wash the plate thoroughly and completely remove any residual wash buffer after each wash cycle. Insufficient washing can lead to erroneously high values and incomplete removal of wash buffer to irregularities due to the dilution of added reagents. As mentioned use a multichannel pipette to add peroxidase conjugate, TMB substrate and stop solution.

### Warning

Potentially biohazardous material. Donor plasma used in this kit was tested by internationally approved methods for the presence of antibodies to HIV and hepatitis B virus and found to be negative. However, all human blood products should be handled as potentially infectious material.

### Literature


### Stand / Date of issue: 11/05