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IMUCLONE® Fibronectin ELISA

Product No. ADG607

Storage: 2–8°C

For Research Use Only!

INTENDED USE

The IMUCLONE® Fibronectin ELISA is an enzyme-linked sandwich immunoassay for the determination of human plasma fibronectin levels, particularly in patients with severe trauma, shock, sepsis, hepatic disease or clotting disorders. The assay is designed to be used with citrated plasma samples and detects only intact fibronectin. The assay is for research use only. It is not intended for diagnostic or therapeutic procedures.

BACKGROUND

Plasma fibronectin is a high molecular weight glycoprotein composed of two nearly identical, 220,000 D polypeptide chains. It is synthesized and secreted by the liver and circulates at a concentration of approximately 300 µg/mL plasma. Fibronectin is considered a "Cell Attachment Protein", with a dimeric structure which allows it to function as a molecular adhesive, holding various molecules together through its binding domains. Binding domains exist for collagen, fibrin, heparin and Staphylococcus aureus. Fibronectin has been shown to play a role in chemotaxis, fibrin clot formation, fibrinolysis, phagocytosis, opsinization and platelet function.

Reduced fibronectin levels are associated with hepatic disorders, septicemia, trauma and are observed during post-operative periods. Fibrin binding is mediated by factor XIII thus fibronectin levels may be reduced by activation of the clotting cascade. Elevated levels may occur during acute phase and pregnancy complications (fibronectin is also considered to be an "Acute Phase Protein"). High fibronectin levels are also associated with several malignant cancers and it may play a role in cell metastasis.

PRINCIPLE

The IMUCLONE Fibronectin ELISA employs a murine monoclonal antibody against human fibronectin capture antibody coated to plastic micro-test wells. Samples incubate in the precoated micro-test wells, extraneous plasma proteins are washed away and a horseradish peroxidase (HRP) conjugated monoclonal antibody is recognizing the bound fibronectin molecules is added, completing the formation of the antibody sandwich complex.

The addition of a 3, 3', 3, 5' - tetramethylbenzidine (TMB) substrate, and its subsequent reaction with the HRP creates a blue colored solution. Sensitivity is increased by addition of a sulfuric acid stop solution, yielding a yellow color. Fibronectin levels are quantified by measuring solution absorbances at 450 nm and comparing the values with those from a standard curve.

REAGENTS

96 antibody coated microwells (12 x 8 well strips)
1 vial Fibronectin Plasma Standard (lyophilized), 100 µg/mL
1 vial Detection Antibody, HRP conjugated anti-human fibronectin antibody
3 vials Dilution Buffer, 0.15M PBS, 1% BSA (20 mL, 2.5x concentrate)
1 vial Substrate, TMB, 12 mL
1 vial Stop Solution, 0.5 M H₂SO₄, 15 mL
1 vial Wash Buffer, 0.15M PBS, 0.05% Tween 20, pH 7.2 (20 mL, 12.5x concentrate)

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

0.22 µm filtered deionized or distilled H₂O (di H₂O)
50-200 µL eight channel multi-pipette, 10-200 µL single pipette
plastic test tubes, laboratory beakers, graduated cylinders
37°C incubator
graph paper
microwell plate washer
microwell plate reader at 450 nm

REAGENT PREPARATION AND STORAGE

- Antibody Coated Microwells:** Once removed from the foil pouch, the microwell strips must be used within 60 minutes. Unused strips may be stored at 2°-8°C until the expiration date when sealed in the original pouch with the desiccant present, protected from any moisture.
- Wash Buffer:** Prepare Wash Buffer by diluting the concentrate 1:12.5 with filtered deionized or distilled H₂O. Add the 20 mL of concentrate to 230 mL of water and mix well. Wash Buffer may be used for up to 4 weeks when stored at 2°-8°C.
- Dilution Buffer:** Prepare Dilution Buffer by diluting the concentrate 1:2.5 with filtered deionized or distilled H₂O. Add the 20 mL of concentrate to 30 mL of water and mix well. Dilution Buffer may be used for up to 4 weeks when stored at 2°-8°C.
- Fibronectin Standard**

Add 0.5 mL of distilled H₂O to the vial of lyophilized plasma standard. Allow to stand for 15 minutes. Mix well.

Use Dilution Buffer to make serial dilutions of the standard. These instructions are for the lot specific standard included in the kit.

Note: Fibronectin is a protein that adheres strongly to glass surfaces. Use plastic or siliconized glass tubes for diluting the standards and plasma samples.

Tube	Standard Concentration	Volume of	Added to
A	2.00 µg/mL	0.02 mL of Standard	0.98 mL Dilution Buffer
B	1.00 µg/mL	0.3 mL from Tube A	0.3 mL Dilution Buffer
C	0.50 µg/mL	0.3 mL from Tube B	0.3 mL Dilution Buffer
D	0.25 µg/mL	0.3 mL from Tube C	0.3 mL Dilution Buffer
E	0.13 µg/mL	0.3 mL from Tube D	0.3 mL Dilution Buffer

The Fibronectin Standard (100 µg/mL) is stable for up to 4 hours when stored at 20°-25°C and stable for up to 8 weeks when aliquoted and stored at -70°C.

WARNING: The Fibronectin Plasma Standard provided in this kit is of human origin. Each donor unit used in the manufacture of this reagent has been tested by an internationally approved method and found to be negative for the presence of antibodies to Hepatitis B surface Antigen (HBsAg) and Human Immunodeficiency Virus (HIV). As no known method can offer complete assurance that products derived from human blood will not transmit HBsAg, HIV or other blood-borne pathogens, this plasma reagent should be handled as recommended for any potentially infectious human serum or blood specimen.

- Detection Antibody:** Prepare sufficient working strength Detection Antibody Conjugate by diluting the concentrate 1:50 with Dilution Buffer. For using only a single 8 well strip, dilute 20 µL of concentrated conjugate into 980 µL of Dilution Buffer. For using all 96 wells, dilute 240 µL of concentrated conjugate into 11.76 mL of Dilution Buffer. Working strength Detection Antibody is stable for up to 1 hour at 20°-25°C.
- Substrate:** Supplied ready to use. The Substrate is stable until the stated expiration date when stored at 2°-8°C.
- Stop Solution:** Supplied ready to use. The Stop Solution is stable until the stated expiration date when stored at 2°-8°C.

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SPECIMEN COLLECTION AND PREPARATION

Citrate collected platelet poor plasma may be used for this assay. See "Collection, Transport and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays", NCCLS Document H21-A3, Vol. 18, No. 20, December 1998. Plasma collection should be performed as follows:

1. Collect 9 parts of blood into 1 part of 3.2% (0.109 M) trisodium citrate anticoagulant solution.
2. Centrifuge the blood sample at 2,000 x g for 15 minutes.
3. Plasma should be stored at 2°-8°C and assayed within 2 hours. Alternatively, plasma may be stored at -20°C for up to 2 weeks or at -70°C for up to 6 months.
4. Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2°- 8°C and assayed within 2 hours.

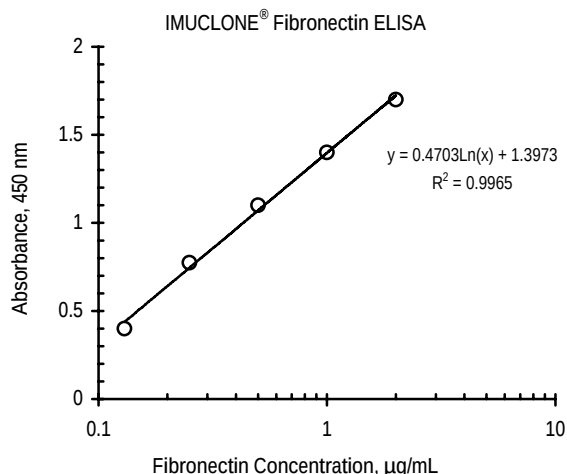
Dilute plasma samples 1:200 to 1:400 with Dilution Buffer. If a sample contains a high fibronectin content, a 1:800 dilution may be appropriate.

ASSAY PROCEDURE

1. Open the foil pouch and remove the frame with the microwell strips. Remove the strips that will not be used and replace in the foil pouch. Tightly reseal the foil pouch and store at 2°-8°C.
2. Wash the microwells 3 times each with 250 µL of Wash Buffer.
3. Add 100 µL of either standard or diluted plasma sample into separate microwells, cover the strips with clear plastic foil and incubate for 1 hour at 37°C. Run standards and samples in duplicate.
4. Wash the microwells 3 times each with 250 µL of Wash buffer.
5. Add 100 µL of working strength Detection Antibody to each microwell, cover and incubate for 30 minutes at 37°C. Discard any unused working strength Detection Antibody.
6. Wash the microwells 3 times each with 250 µL of Wash buffer.
7. Add 100 µL of TMB substrate solution to each microwell, cover and incubate for 15 minutes at 20°-25°C.
8. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each microwell. Read the absorbances on a microwell plate reader at a wavelength of 450 nm within 30 minutes.

REPRESENTATIVE STANDARD CURVE

The standard curve is constructed by plotting the mean absorbance value calculated for each fibronectin standard versus the corresponding fibronectin concentration. Interpolate the fibronectin concentrations for the diluted samples directly from the standard curve. A standard curve should be generated each time the assay is run. The following curve is for demonstration purposes only.



EXPECTED VALUES

The total fibronectin concentration in fresh normal plasma is 330 ± 80 µg/mL. The normal range in plasma (n=50) of the intact form was established at 70-148 µg/mL. In patients with severe septicemia or DIC, fibronectin fragment products are generated by leukocyte or plasmin degradation of the intact molecule. These patients show a decreased level of functional intact fibronectin. There is approximately a 30% loss of fibronectin in freeze-dried plasma.

REFERENCES

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