

## Human Cellular Fibronectin (cFN) ELISA Kit

**Catalog No.:** abx257225

**Size:** 96T

**Range:** 1.563 ng/ml - 100 ng/ml

**Sensitivity:** < 0.938 ng/ml

**Storage:** Store the 96-well plate and standards at -20°C, and the rest of the kit components at 4°C.

**Application:** For quantitative detection of cFN in Human Serum, Plasma, Tissue Homogenates and other biological fluids.

**Introduction:** Human Cellular Fibronectin ELISA Kit is an ELISA Kit against cFN. This kit detects Fibronectin isoforms 7, 11, 13, and 15. Does not detect "canonical" fibronectin.

### Principle of the Assay

This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific to cFN is pre-coated onto a 96-well plate. The standards and test samples are added to the wells and washed with wash buffer. Biotin conjugated antibody specific to cFN is used as a detection antibody. TMB substrate is used to visualize HRP activity. TMB is catalyzed by HRP to produce a blue colour product that changes into yellow after adding stop solution. The intensity of the color yellow is proportional to the cFN amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of cFN can be calculated.

### Kit components

1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Standard: 2 tubes
3. Sample/Standard Diluent Buffer: 20 ml
4. Biotin Conjugated Antibody (Dilution 1:100): 120 µl
5. Antibody Diluent Buffer: 10 ml
6. Streptavidin-HRP Conjugate (Dilution 1:100): 120 µl
7. HRP Diluent Buffer: 10 ml
8. TMB Substrate: 10 ml
9. Stop Solution: 10 ml
10. Wash Buffer (25X): 30 ml
11. Plate Sealer: 5

### Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Absorbent filter papers
8. 100 ml and 1 L volume graduated cylinders

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## Protocol

### A. Preparation of sample and reagents

#### 1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

#### Note:

- » Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C. **Once ready to analyze, thaw samples and dilute at least 1:2.**
- » Samples should be clear and transparent. Samples must be diluted so that the expected concentration falls within the kit's range.
- » Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used. Samples that contain NaN<sub>3</sub> cannot be detected as it interferes with HRP.
- » Always use non-pyrogenic, endotoxin-free tubes for blood collection.

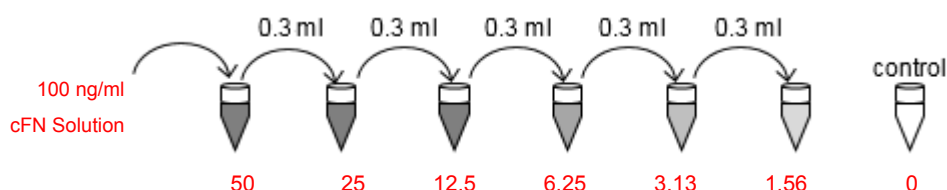
#### 2. Wash buffer

Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

#### 3. Standard

Preparation of the cFN standard: standard solution should be prepared no more than 15 min prior to the experiment. Centrifuge at 10,000×g for 1 minute to spin down the powder.

- 100 ng/ml standard solution. Add 1 ml of Sample/Standard diluent buffer into one Standard tube. Allow the reconstituted standard to sit for 10 minutes with gentle agitation prior to carrying out the serial dilutions; avoiding foaming or bubbles.
- 50 ng/ml → 1.5625 ng/ml standard solutions: Label 6 tubes with 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.125 ng/ml and 1.5625 ng/ml. Aliquot 0.3 ml of the Sample / Standard diluent buffer into each tube. Add 0.3 ml of the above 100 ng/ml standard solution into 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix thoroughly, and so on.



**Note:** Do not vortex the standard during reconstitution, as this will destabilize the protein. Once your standard has been reconstituted,

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it should be used right away. Use the diluted Standards for a single assay procedure and discard after use.

## 4. Preparation of Biotin conjugated antibody working solution: prepare no more than 1 hour before the experiment.

- a.) Calculate the total volume of the working solution:  $0.1 \text{ ml / well} \times \text{quantity of wells}$ . (Allow 0.1-0.2 ml more than the total volume).
- b.) Dilute the Biotin conjugated antibody with antibody diluent buffer at 1/100 and mix thoroughly. i.e. Add 1  $\mu\text{l}$  of Biotin conjugated antibody into 99  $\mu\text{l}$  of antibody diluent buffer.

## 5. Preparation of Streptavidin-HRP working solution: prepare no more than 30 min. before the experiment

- a.) Calculate the total volume of the working solution:  $0.1 \text{ ml / well} \times \text{quantity of wells}$ . (Allow 0.1-0.2 ml more than the total volume).
- b.) Dilute the Streptavidin-HRP Conjugate with HRP diluent buffer at 1/100 and mix thoroughly. i.e. Add 1  $\mu\text{l}$  of Streptavidin-HRP Conjugate into 99  $\mu\text{l}$  of HRP diluent buffer.

## B. Assay Procedure

Equilibrate the TMB substrate at 37°C for 30 minutes prior to use. It is recommended to plot a standard curve for each test.

1. **Wash the plate two times with 1X Wash Buffer before adding standard, samples and buffers.** Fill each well completely with Wash Buffer (350  $\mu\text{l}$ ) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels. Any strips that are not being used should be kept dry and stored at 4°C. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate. Mix the standards and samples up and down to be homogeneous before adding into the wells but avoid adding bubbles. Add the solution at the bottom of each well without touching the side walls.
2. Add 100  $\mu\text{l}$  of the prepared standard solutions into the standard wells.
3. Add 100  $\mu\text{l}$  of Sample / Standard diluent buffer into the control (zero) well.
4. Add 100  $\mu\text{l}$  of appropriately diluted sample into test sample wells.
5. Cover the plate and incubate at 37°C for 90 minutes.
6. Remove the cover and discard the liquid. Wash the plate two times with 1X Wash Buffer.
7. Add 100  $\mu\text{l}$  of prepared Biotin conjugated antibody working solution into each well (standard, test sample and zero well). Add the solution at the bottom of each well without touching the side walls. Seal the plate with a cover and incubate at 37°C for 60 minutes.
8. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer.
9. Add 100  $\mu\text{l}$  of Streptavidin-HRP working solution into each well, cover the plate and incubate at 37°C for 30 minutes.
10. Remove the cover and wash the plate 5 times with 1X Wash Buffer.
11. Add 90  $\mu\text{l}$  of TMB Substrate into each well. Seal the plate with a cover and incubate at 37°C for 10-20 min. Avoid exposure to light. The incubation time is for reference only, the optimal time should be determined by end user. Do not exceed 30 min.
12. Add 50  $\mu\text{l}$  of Stop solution into each well to stop the enzyme reaction. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
13. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

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For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). Log-log curve fitting is recommended for data analysis. The Human cFN concentration of the samples can be interpolated from the standard curve.

**Note:** If the samples measured were diluted, multiply the dilution factor by the concentrations from interpolation to obtain the concentration before dilution.

## C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
3. If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
4. Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard dilutions within 15 min of use and discard any unused working standards. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
6. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells, avoid letting the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
7. Ensure plates are properly sealed or covered during incubation steps.
8. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
9. To avoid cross contamination do not reuse pipette tips and tubes.
10. Do not use components from a different kit or expired ones.
11. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. The TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

## D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of cFN were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of cFN were tested on 3 different plates, 8 replicates in each plate.

$$CV (\%) = (\text{Standard Deviation} / \text{mean}) \times 100$$

Intra-Assay: CV<8%

Inter-Assay: CV<10%

## E. Typical Data & Standard Curve

Typical Standard Curve Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

| Concentration<br>ng/ml | 0     | 1.5625 | 3.125 | 6.25  | 12.5  | 25    | 50    | 100   |
|------------------------|-------|--------|-------|-------|-------|-------|-------|-------|
| OD450                  | 0.074 | 0.213  | 0.351 | 0.453 | 1.014 | 1.625 | 2.147 | 2.625 |

