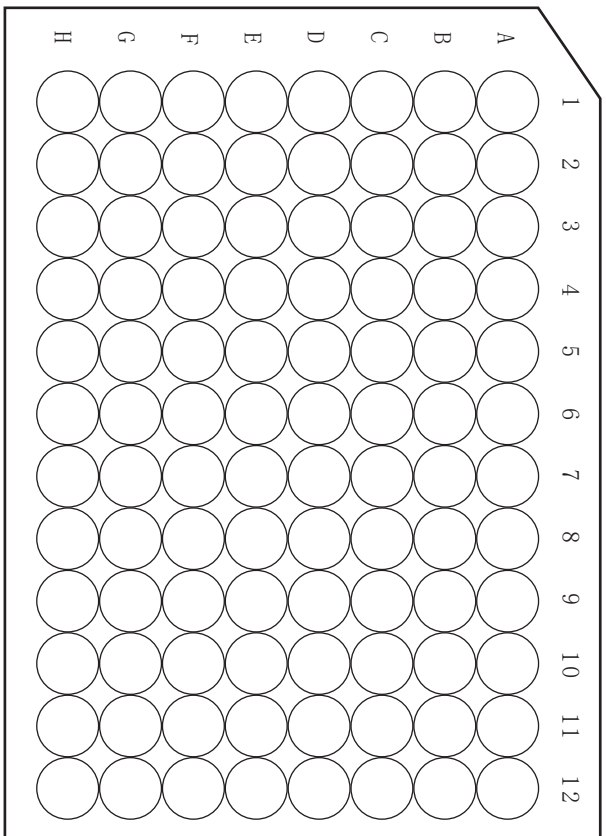

Human Fibronectin Immunoassay

Catalog Number:SEKH-0516

For the quantitative determination of human Fibronectin concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of Fibronectin in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

The linearity of the assay

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	93	92
	Range(%)	85-104	83-101
1:4	Average% of Expected	95	93
	Range(%)	90-107	88-103

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 0.78ng/mL.
SPECIFICITY: This assay recognizes both natural and recombinant human Fibronectin. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
RECOVERY: The recovery of Fibronectin spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of Fibronectin in two matrices

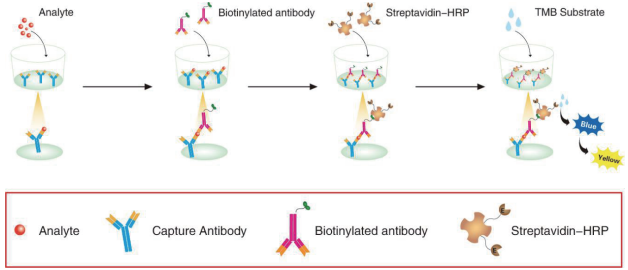
Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	92	88-106
Cell culture supernatants	97	83-104

BACKGROUND

Fibronectin is an extracellular matrix (ECM) component and is one of the primary cell adhesion molecules. The occurrence of different isoforms is due to alternative mRNA splicing of the ED-A, ED-B and III-CS regions, and subsequent post-translational modification. Although non-reactive with adhesion receptors in its soluble state, fibronectin is highly adhesive when immobilized on a surface.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Fibronectin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Fibronectin present is captured by the coated antibody after incubation. Following extensive washing, a HRP-conjugated antibody specific for Fibronectin is added to detect the captured Fibronectin protein in sample. The wells are then washed to remove unbound HRP-labled antibody and Tetramethyl-benzidine (TMB) reagent is added. Incubated at room temperature, only those wells that contain Fibronectin, HRP-labled antibody will appear blue in color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450nm. The OD value is proportional to the concentration of human Fibronectin. You can calculate the concentration of human Fibronectin in the sample by comparing the OD of the sample to the standard cure



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

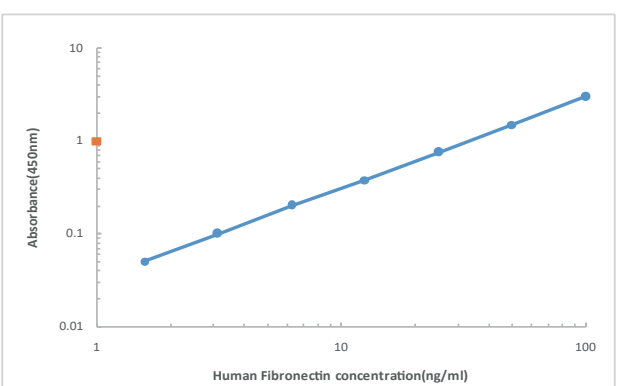
PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5.This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the Fibronectin ELISA

Standared(ng/ml)	OD.	OD.	Average	Corrected
0	0.048	0.054	0.051	--
1.56	0.200	0.203	0.201	0.150
3.12	0.292	0.294	0.293	0.242
6.25	0.434	0.438	0.436	0.385
12.5	0.689	0.696	0.692	0.641
25	1.118	1.130	1.124	1.073
50	1.821	1.839	1.830	1.779
100	2.967	2.997	2.982	2.931



Representative standard curve for Fibronectin **ELISA.**

ASSAY PROCEDURE

Prepare all reagents and standards as directed.Wash three times before assay.

⇩

Add 100µl standard or samples to each well, incubate 90 minutes, 37℃.

⇩ Aspirate and wash 4

Add 100µl working solution of Biotin-Conjugate anti-human Fibronectin antibody to each well, incubate 60 minutes, 37℃.

⇩ Aspirate and wash 4

Add 100µl working solution of Streptavidin-HRP to each well, incubate 30 minutes, 37℃.

⇩ Aspirate and wash 5

Add 100µl Substrate solution to each well, incubate 15 minutes, 37℃. Protect from light.

⇩

Add 50µl Stop solution to each well. Read at 450nm within 30 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the Fibronectin concentrations versus the log of the O.D. and the best fit line can be

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard-lyophilized, 100ng/ml upon reconstitution	2 vials	Store at 2-8°C **for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP (100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent- 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent- 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent- 16 ml/vial	1 bottle	Store at 2-8°C **for six months
20 x Wash Buffer Concentrate- 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution- 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

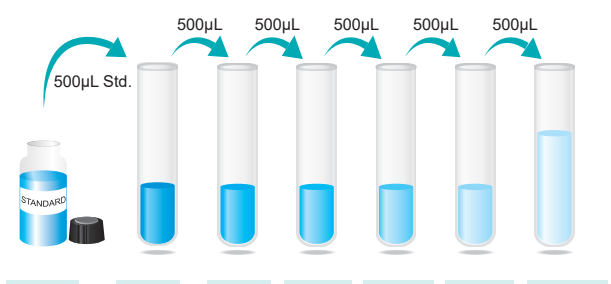
SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000g (or 3000rpm) to remove debris. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃.Centrifuge approximately for 15 minutes at 1000g(or 3000rpm). Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Plasma -Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000g(or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature(20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard**Sample - Reconstitute the Standard with 1mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 100ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500µL of Standard/Sample Diluent into 50ng/ml tube and the remaining tubes. Use the stock solution of 100ng/mL to produce a 2-folddilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 100ng/mL standard serves as the high standard. TheStandard/Sample Diluent serves as the zero standard (0 pg/mL).
   ***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**
4. **Working solution of Biotin-Conjugate anti-human** Fibronectin **antibody:-** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**
5. **Working solution of Streptavidin-HRP:** Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.



Preparation of Fibronectin **standard**