

PRODUCT DESCRIPTION

Fibronectin is an extracellular matrix protein that is found abundantly in blood, connective tissues, and remodeled matrices that are associated with epithelial-to-mesenchymal transition of migratory cells, including tumor cells with stem cell-like properties (1-3). Fibronectin performs essential functions in collagen fibrillogenesis, general cell adhesion, and as modulator in binding between cell surfaces and the extracellular matrix (2,4). Fibronectin matrix assembly is essential for normal vertebrate development and contributes to the generation of tumor metastases by supporting the establishment and persistence of pre-metastatic niches (2,5,6). Fibronectin is secreted as a disulfide-linked dimer of 230-270 kDa and is comprised of three types of repeating modules that mediate interactions with extracellular matrix components (including fibronectin itself) as well as cells (via integrins and other fibronectin receptors) (2).

INTENDED USE

Cultrex Human Fibronectin can be used for coating tissue culture surfaces or as a medium additive to promote cell adhesion and proliferation.

PRODUCT SPECIFICATIONS

Concentration	1 mg/mL
Source	Human plasma
Storage Buffer	100 mM CAPS, 150 mM NaCl, 1 mM CaCl ₂ , pH 11.5.
Stability	Product is stable for a minimum of 3 months from date of shipment when stored at ≤ -20 °C. For optimal stability store at ≤ -70 °C. Avoid freeze-thaw cycles.
Storage	Store at ≤ -70 °C.

PRECAUTION

When handling bio-hazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

Cultrex Human Fibronectin is purified from human source material and therefore should be treated as potentially infectious and handled at Biological Safety Level 2 to minimize exposure.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- Results may vary due to variations among tissue/cells derived from different donors or sources.

MATERIAL QUALIFICATIONS

Sterility Testing:

- PathClear® - Tested negative by PCR test for mycoplasma and human pathogenic viruses, including: EBV, HAdV, Hantaan, HCMV, Hepatitis A, Hepatitis B, Hepatitis C, HHV 6, HHV 8, HIV1, HIV2, HSV 1, HSV 2, HTLV 1, HTLV 2, LCMV, Seoul, Sin Nombre, and VZV.
- Tested following USP <71> sterility guidelines.
- Endotoxin concentration < 20 EU/ml by LAL assay.

Functional Assays:

- Tested for ability to promote attachment of HT-1080 cells.

COATING PROCEDURES

The recommended working concentration is 0.2-2.0 µg/cm² (1-10 µg/mL) of growth surface, depending on cell type. Only dilute as much Cultrex Human Fibronectin as needed and use immediately.

Note: *Cultrex Human Fibronectin is not stable for extended periods of time when diluted. Store concentrated material in aliquots to reduce the number of freeze-thaw cycles.*

1. Dilute Cultrex Human Fibronectin stock appropriately with sterile water. Mix thoroughly.
2. Pipette the appropriate amount of solution into each well of the tissue culture plate (Table 1). Spread the solution to completely cover the bottom of the wells.

PLATE TYPE	CULTREX HUMAN FIBRONECTIN (VOLUME/WELL)
6 wells (or 35 mm dish)	2.0 mL
24 wells	600 µL
48 wells	200 µL
96 wells	100 µL

Table 1: Suggested plating volumes for Cultrex Human Fibronectin plate-coating.

3. Incubate at 37 °C overnight.
4. Aspirate solution and rinse the wells once with sterile water.
5. Block wells using Phosphate Buffered Saline (PBS) containing 2% BSA for one hour at 37 °C.
6. Immediately add cells to wells (optimize concentration for each cell line and experimental condition). **Do not allow coated surface to dry out.**
7. Culture and analyze cells as needed (optimize for each cell line and experimental condition).

REFERENCES

1. Vaheri, A. and D.F. Mosher (1978) *Biochim. Biophys. Acta.* **516**:1.
2. Mao, Y. and J.E. Schwarzbauer (2005) *Matrix Biol.* **24**:389.
3. Polyak, K. and R.A. Weinberg (2009) *Nat. Rev. Cancer* **9**:265.
4. Kadler, K.E. *et al.* (2008) *Curr. Opin. Cell Biol.* **20**:495.
5. Hunt, G.C. and J. E. Schwarzbauer (2009) *Developmental Cell* **16**:327.
6. Psaila, B. and D. Lyden (2009) *Nat. Rev. Cancer* **9**:285.