

# CULTREX<sup>®</sup> Product Data

*For Research Use Only. Not For Use In Diagnostic Procedures*

## Cultrex<sup>®</sup> Stem Cell Qualified Human Fibronectin, PathClear<sup>®</sup>\*

**Catalog #:** 3420-001-03

**Size:** 1 mg

**Description:** Fibronectin provides a functionally defined and effective feeder-free surface for the attachment and maintenance of embryonic stem cells in a pluripotent state (fig. 1).<sup>1</sup> It is an extracellular matrix protein that is found abundantly in blood, connective tissues, and remodeled matrices also associated with the epithelial to mesenchymal transition of migratory cells—including tumor cells with stem cell-like properties.<sup>2,4</sup> Fibronectin performs essential functions in collagen fibrillogenesis, and as either a general cell adhesion molecule or as a modulator in binding between cell surfaces and the extracellular matrix.<sup>2,5</sup> Fibronectin matrix assembly is essential for normal vertebrate development, and apparently contributes to the generation of tumor metastases by supporting the establishment and persistence of pre-metastatic niches.<sup>3,6</sup> Fibronectin is secreted as a disulfide-linked dimer of 230-270 kDa, comprised of three types of repeating modules that mediate interactions with extracellular matrix components (including fibronectin itself), and cells via integrins and other fibronectin receptors.<sup>3</sup>

### Specifications:

**Concentration:** 1 mg/ml  
**Purity:** Purity >90% by SDS-PAGE.  
**Source:** Human plasma.  
**Storage Buffer:** 100 mM CAPS, 150 mM NaCl, 1 mM CaCl<sub>2</sub>, pH 11.5  
**Storage/Stability:** Product is stable for a minimum of 3 months from date of shipment when stored at -20°C. For optimal stability store at -80°C. **Repeated freeze-thaws will destroy product integrity.**

### Materials Qualification:

#### Functional Assay:

- Promotes the attachment of H9 human embryonic stem cells.
- Effectively maintains human embryonic stem cells in a pluripotent state as evidenced by intracellular staining for the stem cell markers Oct-4 and Nanog.

#### \*Sterility Testing:

- No bacterial or fungal growth detected after incubation at 37°C for 14 days following USP sterility testing protocols guidelines.
- No mycoplasma contamination detected by PCR.
- Stem Cell Qualified Human Fibronectin, PathClear<sup>®</sup> is tested negative by PCR for different human pathogenic viruses including EBV, HAAdV, Hantaan, HCMV, Hepatitis A, Hepatitis B, Hepatitis C, HHV 6, HHV 8, IV1, HIV2, HSV 1, HSV 2, HTLV 1, HTLV 2, LCMV, Seoul, Sin Nombre, VZV .
- Endotoxin concentration < 20 EU/ml by LAL assay.

**Safety Statement:** Cultrex<sup>®</sup> Stem Cell Qualified Human Fibronectin, PathClear<sup>®</sup> is purified from human source material and therefore should be treated as potentially infectious and handled at Biological Safety Level 2 to minimize exposure.

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# TREVIGEN<sup>®</sup>

8405 Helgerman Court, Gaithersburg, MD 20877 USA

Voice: 1-800-TREVIGEN (1-800-873-8443) • 301-216-2800

Fax: 301-560-4973 • e-mail: info@trevigen.com • www.trevigen.com

### Coating procedure for Stem Cell Propagation:

The recommended working concentration is 5  $\mu\text{g}/\text{cm}^2$  of growth surface depending on cell type. Empirical determination of the optimal coating concentration for your application may be required.

1. Thaw Human Fibronectin on ice for several hours.
2. In a laminar flow hood, dilute to a final concentration 50  $\mu\text{g}/\text{ml}$  with serum-free cell culture medium.
3. Mix and transfer to the wells of tissue culture plates. Spread the solution to completely cover the bottom of the wells.
4. Incubate coated object at room temperature for an hour.
5. Aspirate coating solution and immediately plate cells. **Do not allow coated surface to dry out.**

The following table is a guide for the suggested volumes required per well:

<u>Plate type/</u>	<u>Volume Fibronectin per Well</u>
6 wells (or 35 mm dish)	1 – 1.5 ml
12 wells	500 - 600 $\mu\text{l}$
24 wells	250 – 300 $\mu\text{l}$
48 wells	150 $\mu\text{l}$
96 wells	50 $\mu\text{l}$

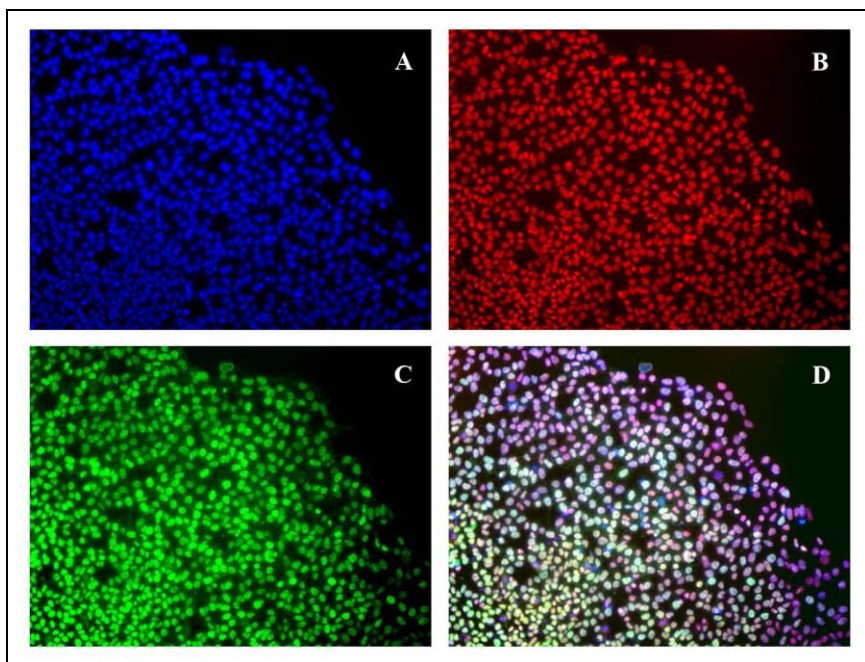


Fig.1. H9 human embryonic stem cells after three passages on Cultrex<sup>®</sup> Stem Cell Qualified Human Fibronectin, PathClear<sup>®</sup> maintain expression of the non-differentiated stem cell markers Sox 2 (B) and Oct-4 (C). Nuclear staining by DAPI shown on panel (A) and merged image shown on panel (D).

Images courtesy of the Yanik lab, MIT <http://www.rle.mit.edu/bbng>

**References:**

1. Amit M, Shariki C, Margulets V, Itskovitz-Eldor J. (2004). Feeder layer- and serum-free culture of human embryonic stem cells. *Biology of Reproduction*, 70:837-45.
2. Vaheiri A, Mosher DF. (1978) High molecular weight, cell surface-associated glyco-protein (fibronectin) lost in malignant transformation. *Biochim Biophys Acta*. 516:1-25.
3. Mao Y, Schwarzbauer JE. (2005) Fibronectin fibrillogenesis, a cell-mediated matrix assembly process. *Matrix Biol* 24:389-99.
4. Polyak K, Weinberg RA. (2009) Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. *Nat Rev Cancer* 9:265-73.
5. Kadler KE, Hill A, Canty-Laird EG. (2008) Collagen fibrillogenesis: fibronectin, integrins, and minor collagens as organizers and nucleators. *Curr Opin Cell Biol* 20:495-501.
6. Hunt GC, Schwarzbauer JE. (2009) Tightening the connections between cadherins and fibronectin matrix. *Developmental Cell* 16:327-8.

**Related Products:**

Catalog#	Description	Size
3400-010-03	Cultrex® Stem Cell Qualified Laminin I, PathClear®	1 mg
3421-001-03	Cultrex® Stem Cell Qualified Human Vitronectin, PathClear®	200 µg
3415-001-03	Cultrex® Stem Cell Qualified Human BME, PathClear®	1 ml
3434-005-02	Cultrex® Stem Cell Qualified BME, Growth Factor Reduced PathClear®	5 ml



**Stem Cell Qualified  
Human Fibronectin,  
PathClear<sup>®</sup>**

Catalog #: 3420-001-03

Storage: -80 °C

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**1-800-873-8443**