



Cultrex® Stem Cell Qualified Human Vitronectin, *PathClear®

Catalog #: 3421-001-03

Size: 200 µg

Description: Vitronectin provides a functionally defined and effective feeder-free surface for the attachment and maintenance of embryonic stem cells in a pluripotent state (fig. 1).¹ It is an extracellular, soluble, disulfide-linked dimer, composed of a 75 kDa and a 65 kDa peptide chain with a total molecular weight of 140 kDa. Vitronectin is a major plasma glycoprotein that promotes cellular adhesion and spreading,^{2,3} inhibits the membrane-damaging effect of the terminal cytolytic complement pathway,⁴ and binds to several serpin serine protease inhibitors.^{5,6} Vitronectin, along with collagen IV, fibronectin, and laminin can support robust, long term proliferation of human embryonic stem cells in the undifferentiated state.^{1,7} Vitronectin can be used for coating tissue culture surfaces to promote cell adhesion, proliferation and differentiation, or as an additive for serum-free media. Trevigen's Stem Cell Qualified Human Vitronectin has a reduced nucleic acid content.

Specifications:

Concentration: ~1 mg/ml (Please see lot specific data.)

Source: Human plasma

Storage Buffer: 10 mM Sodium Phosphate, pH 7.7, 8 M Urea, 5 mM EDTA, 0.5 M NaCl.

Storage/Stability: Product is stable for a minimum of 3 months from date of shipment when stored 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 guidelines.
- No mycoplasma contamination detected by PCR.
- Stem Cell Qualified Human Vitronectin, PathClear® is tested negative by PCR for different 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, VZV.
- Endotoxin concentration < 20 EU/ml by LAL assay.

Safety Statement: Cultrex® Human Vitronectin, PathClear® is purified from human source material and therefore should be treated as potentially infectious and handled at Biological Safety Level 2 to minimize exposure.

Coating procedure for Stem Cell Propagation:

The recommended working concentration is $1 \mu\text{g}/\text{cm}^2$ of growth surface depending on cell type.

1. Thaw human vitronectin on ice for several hours.
2. In a laminar flow hood, dilute to $10 \mu\text{g}/\text{ml}$ with cold 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 Vitronectin per Well</u>
6 wells (or 35 mm dish)	1 ml
24 wells	0.5 ml
48 wells	100 μl
96 wells	50 μl

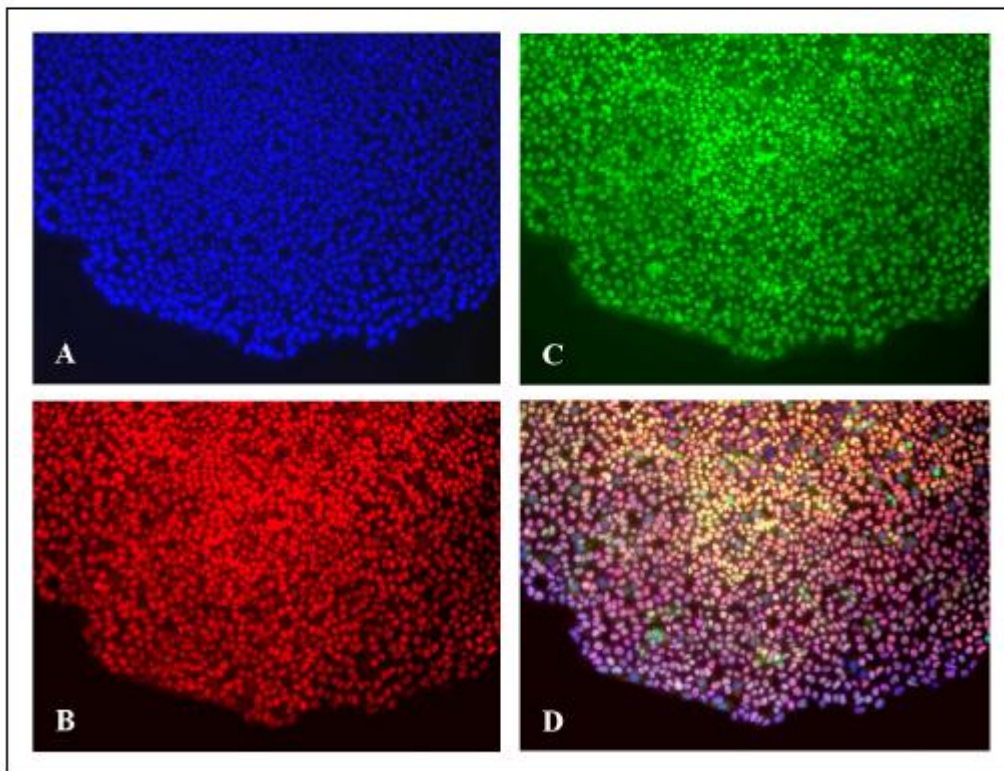


Fig.1. H9 human embryonic stem cells after three passages on Cultrex[®] Stem Cell Qualified Human Vitronectin, PathClear[®] maintain expression of the non-differentiated stem cell markers Oct-4 (B) and Nanog (C). Nuclear staining by DAPI shown on panel (A) and merged image shown on panel (D).

References:

1. Braam SR, Zeinstra L, Litjens S, Ward-van Oostwaard D, van den Brink S, van Laake L, Lebrin F, Kats P, Hochstenbach R, Passier R, Sonnenberg A, Mummery CL. (2008) Recombinant vitronectin is a functionally defined substrate that supports human embryonic stem cell self-renewal via $\alpha V\beta 5$ integrin. *Stem Cells*. 26:2257-65.
2. Vuento M, Korkkolainen M, Kuusela P, Holttä E. (1985) Isolation of a novel cell-attachment and spreading-promoting protein from human serum. *Biochem J*. 227:421-7.
3. Hayman EG, Pierschbacher MD, Suzuki S, Ruoslahti E (1985). Vitronectin--a major cell attachment promoting protein in fetal bovine serum. *Exp Cell Res*.160:245-58.
4. Tschopp J, Masson D, Schafer S, Peitsch M, Preissner KT (1988). The heparin binding domain of S protein/vitronectin binds to complement components C7, C8, and C9 and perforin from cytolytic T cells and inhibits their lytic activities. *Biochemistry* 27:4103-9.
5. Wiman B, Almquist A, Sigurdardottir O, Lindahl T. (1988) Plasminogen activator inhibitor 1 (PAI) is bound to vitronectin in plasma. *FEBS Lett*. 242:125-8.
6. Czekay RP, Aertgeerts K, Curriden SA, Loskutoff DJ. (2003) Plasminogen activator inhibitor-1 detaches cells from extracellular matrices by inactivating integrins. *J Cell Biol*. 160:781-91.
7. Ludwig TE, Levenstein ME, Jones JM, Berggren WT, Mitchen ER, Frane JL, Crandall LJ, Daigh CA, Conrad KR, Piekarczyk MS, Llanas RA, Thomson JA. (2005) Derivation of human embryonic stem cells in defined conditions. *Nat Biotech*. 24:185-7.

Related Products:

Catalog#	Description	Size
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
3400-010-03	Cultrex [®] Stem Cell Qualified Laminin I, PathClear [®]	1 mg
3420-001-03	Cultrex [®] Stem Cell Qualified Human Fibronectin, PathClear [®]	1 mg

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