Cultrex® Stem Cell Qualified Human Vitronectin, PathClear®

Catalog Number: 3421-001-03 (200 µg)

PRODUCT DESCRIPTION

Vitronectin provides a functionally defined and effective feeder-free surface for the attachment and maintenance of embryonic stem cells in a pluripotent state (Figure 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.

INTENDED USE

Cultrex Stem Cell Qualified Human Vitronectin is a purified stromal ECM protein that has been developed, produced, and qualified as a coating for cell attachment. Cultrex Stem Cell Qualified Human Vitronectin is an extracellular matrix coating solution that provides a functionally defined and effective feeder-free surface for the attachment and maintenance of embryonic stem cells in a pluripotent state. Cultrex Stem Cell Qualified Human Vitronectin may also be used as to supplement customized medium formulations for cell culture.

PRODUCT SPECIFICATIONS

Concentration	1 mg/mL
Source	Human plasma
Storage Buffer	10 mM Sodium Phosphate, pH 7.7, 8 M Urea, 5 mM EDTA, 0.5 M NaCl.
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.

PRECAUTIONS

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

Cultrex® Stem Cell Qualified Human Vitronectin 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:

- No bacterial of fungal growth detected after a 14 day culture.
- No mycoplasma contamination detected by PCR.
- Tested negative by PCR for 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.
- Endotoxin concentration < 20 EU/mL by LAL assay.

Functional Assays:

- 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.

COATING PROCEDURES FOR STEM CELLS CULTURE

The recommended working concentration is 1 µg/cm^2 of growth surface, depending on cell type. Empirical determination of the optimal coating concentration for your application may be required.

1. Quickly thaw Cultrex Stem Cell Qualified Human Vitronectin at room temperature.
2. In a laminar flow hood, dilute to a final concentration 10 µg/mL with serum-free cell culture medium.
3. Mix Cultrex Stem Cell Qualified Human Vitronectin and transfer into desired tissue culture plates. Add a sufficient amount of volume to cover the entire growth surface area (Table 1). Spread the solution to completely cover the bottom of the wells.

PLATE TYPE	CULTREX STEM CELL QUALIFIED HUMAN VITRONECTIN (VOLUME/WELL)
6 wells (or 35 mm dish)	1-1.5 mL
12 wells	500-600 µL
24 wells	250-300 µL
48 wells	150 µL
96 wells	50 µL

Table 1: Suggested plating volumes for Cultrex Stem Cell Qualified Human Vitronectin plate-coating.

4. Incubate coated plates at room temperature for an hour.
5. Aspirate coating solution and immediately plate cells. **Do not allow coated surface to dry out.**

DATA EXAMPLE

Figure 1: Verification of Stemness of Human Embryonic Stem Cells Cultured Cultrex Stem Cell Qualified Human Vitronectin, PathClear®. H9 human embryonic stem cells were cultured for three passages on Cultrex Stem Cell Qualified Human Vitronectin. Cells 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)**. Images courtesy of the Yanik lab, MIT.

REFERENCES

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