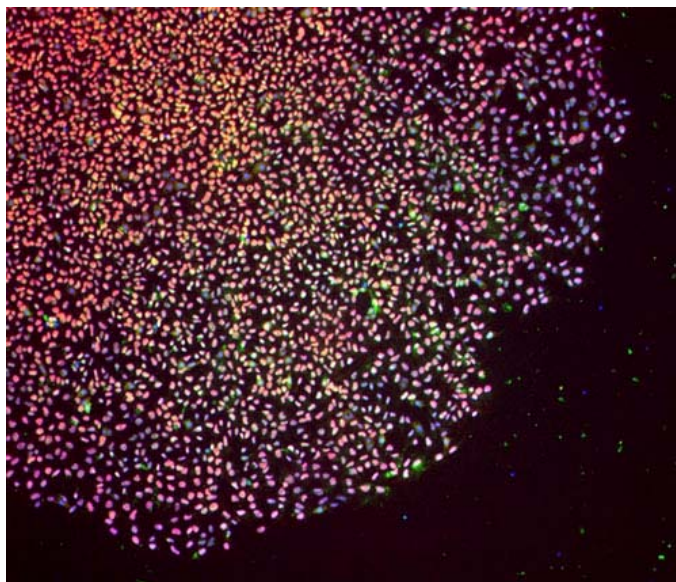


Cultrex[®] Stem Cell Qualified Protein Set
Catalog #: 3434-SCQ-K



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I. Background

Trevigen's Cultrex® Stem Cell Qualified Protein Set was created to help researchers in choosing optimal coating conditions for feeder-free culture of stem cells of interest.

Cultrex® Stem Cell Qualified Protein Set contains 5 different extracellular matrix proteins: Human Basement Membrane Extract (BME); Human Fibronectin; Human Vitronectin; Mouse Reduced Growth Factor BME and Mouse Laminin I. Each of these proteins was qualified in feeder-free cell culture to support human embryonic stem cells in the undifferentiated state for multiple passages.

II. Materials Supplied

| <u>Component</u> | <u>Quantity</u> | <u>Storage</u> | <u>Catalog#</u> |
|---|-----------------|----------------|-----------------|
| Stem Cell Qualified Human BME, PathClear® | 1 ml | -20°C / -80°C | 3415-001-03 |
| Stem Cell Qualified Human Fibronectin, PathClear® | 1 ml | -20°C / -80°C | 3420-001-03 |
| Stem Cell Qualified Human Vitronectin, PathClear® | 200 µl | -20°C / -80°C | 3421-001-03 |
| Stem Cell Qualified BME Growth Factor Reduced, PathClear® | 1 ml | -20°C / -80°C | 3434-001-02 |
| Stem Cell Qualified Laminin I, PathClear® | 1 ml | -20°C / -80°C | 3400-010-03 |

III. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. The physical, chemical, and toxicological properties of these products may not yet have been fully investigated; therefore, Trevigen recommends the use of gloves, lab coats, and eye protection while using these chemical reagents. Trevigen assumes no liability for damage resulting from handling or contact with these products.
3. Cultrex® Stem Cell Qualified Human BME, Human Fibronectin, and Human Vitronectin, are obtained from human source material and therefore should be treated as potentially infectious and handled at Biological Safety Level 2 to minimize exposure. All Cultrex® Stem Cell Qualified Protein Set reagents may be harmful if swallowed, or upon contact with skin or eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Material safety data sheets are available on request.

IV. Individual Product Descriptions and Information

Cultrex® Stem Cell Qualified Human BME, PathClear®

Catalog #: 3415-001-03

Size: 1 ml

Description: Basement membranes are continuous sheets of specialized extracellular matrix that form an interface between endothelial, epithelial, muscle, or neuronal cells and their adjacent stroma. Basement membranes are degraded and regenerated during development and wound repair. They not only support cells and cell layers, but they also play an essential role in tissue organization that affects cell adhesion, migration, proliferation, and differentiation. Basement membranes provide major barriers to invasion by metastatic tumor cells. Cultrex® Human Basement Membrane Extract (BME) is a soluble form of basement membrane purified from human placenta. BME can be used for promotion and maintenance of an undifferentiated phenotype, or for the directed differentiation of precursor cells, under a variety of cell culture conditions, including stem cells, primary epithelial cells, endothelial cells and smooth muscle cells.

Specifications:

Concentration: 1 mg/ml

Source: Human Placenta

Storage Buffer: Dulbecco's Modified Eagle's medium containing 10 µg/ml gentamicin sulfate and no phenol red.

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 guidelines.
- No mycoplasma contamination detected by PCR.
- Stem Cell Qualified Human BME, 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 and VZV.
- Endotoxin concentration < 20 EU/ml by LAL assay.

Cultrex® Stem Cell Qualified Human Fibronectin, PathClear®

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). 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. 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. 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. 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.

Specifications:

Concentration: 1 mg/ml

Source: Human plasma.

Storage Buffer: 100 mM CAPS, 150 mM NaCl, 1 mM CaCl₂, 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 guidelines.
- No mycoplasma contamination detected by PCR.
- Stem Cell Qualified Human Fibronectin, 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 and VZV.
- Endotoxin concentration < 20 EU/ml by LAL assay.

Cultrex® Stem Cell Qualified Human Vitronectin, PathClear®

Catalog #: 3421-001-03

Size: 200 µl

Description: Vitronectin provides a functionally defined and effective feeder-free surface for the attachment and maintenance of embryonic stem cells in a pluripotent state. 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, inhibits the membrane-damaging effect of the terminal cytolytic complement pathway, and binds to several serpin serine protease inhibitors. Vitronectin, along with collagen IV, fibronectin, and laminin can support robust, long term proliferation of human embryonic stem cells in the undifferentiated state. 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 reduced nucleic acid content.

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.

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 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 and VZV.
- Endotoxin concentration < 20 EU/ml by LAL assay.

Cultrex® Stem Cell Qualified BME, Growth Factor Reduced, PathClear®

Catalog #: 3434-001-02

Size: 1 ml

Description: Cultrex® Stem Cell Qualified Basement Membrane Extract (BME) has been shown to provide an effective feeder-free surface for the attachment and maintenance of human and mouse embryonic stem cells in a pluripotent state, thereby enabling its use for growth promotion or study of stem cell differentiation.

Basement membranes are continuous sheets of specialized extracellular matrix that form an interface between endothelial, epithelial, muscle, or neuronal cells and their adjacent stroma. Basement membranes are degraded and regenerated during development and wound repair. They not only support cells and cell layers, but they also play an essential role in tissue organization that affects cell adhesion, migration, proliferation, and differentiation. Basement membranes provide major barriers to invasion by metastatic tumor cells. Cultrex® BME is a soluble form of basement membrane purified from Engelbreth-Holm-Swarm (EHS) tumor. The major components of BME include laminin, collagen IV, entactin, and heparin sulfate proteoglycan.

Specifications:

Concentration: 12 - 18 mg/ml (see lot specific information on the label)

Source: Murine Engelbreth-Holm-Swarm (EHS) tumor

Storage Buffer: Dulbecco's Modified Eagle's medium with 10 µg/ml gentamycin sulfate and without phenol red.

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.**

Material 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.
- Endotoxin concentrations ≤ 8 EU/ml by LAL assay.
- Cultrex® Stem Cell Qualified Basement Membrane Extract, PathClear® is tested negative by PCR for mycoplasma, 17 bacterial and virus strains typically included in mouse antibody production (MAP) testing, plus 13 additional murine infectious agents including LDEV, for a total of 31 organisms and viruses.

Cultrex® Stem Cell Qualified Laminin I, PathClear®

Catalog #: 3400-010-03

Size: 1 ml

Description: Cultrex® Stem Cell Qualified Laminin I, PathClear® provides a functionally defined and effective feeder-free surface for the attachment and maintenance of embryonic stem cells in a pluripotent state, thereby enabling its use for growth promotion or study of stem cell differentiation. This highly purified preparation of mouse Laminin I is composed of α1β1γ1 chains with a total Mr of 800,000.

Specifications:

Concentration: 1 mg/ml

Source: Murine Engelbreth-Holm-Swarm (EHS) tumor.

Purity: Purity >90% by SDS-PAGE.

Storage Buffer: Dulbecco's Modified Eagle's medium with 10 µg/ml gentamycin sulfate and without phenol red.

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.**

Material 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.
- Endotoxin concentrations < 20 EU/ml by LAL assay.
- Cultrex® Stem Cell Qualified Laminin I, PathClear® is tested negative by PCR test for: mycoplasma, 17 bacterial and virus strains typically included in mouse antibody production (MAP) testing, plus 13 additional murine infectious agents including LDEV, for a total of 31 organisms and viruses.

V. Coating procedure for Stem Cell Propagation (Thaw reagents completely before diluting!)

Empirical determination of the optimal coating concentration for your application may be required.

Table 1: The recommended working concentrations:

| Product name | Catalog # | Concentration | Working concentration |
|----------------------------|-------------|---|-----------------------|
| Human BME | 3415-001-03 | 1 mg/ml | 100 µg/ml |
| Human Fibronectin | 3420-001-03 | 1 mg/ml | 50 µg/ml |
| Human Vitronectin | 3421-001-03 | 1 mg/ml | 10 µg/ml |
| BME, Growth Factor Reduced | 3434-001-02 | 12 – 17 mg/ml *See lot specific information on the label | 150 µg/ml |
| Laminin I | 3400-010-03 | 1 mg/ml | 100 µg/ml |

1. Thaw protein of interest on ice for several hours,
Note: Human Vitronectin should be quickly thawed at room temperature.
2. In a laminar flow hood, prepare coating solution at recommended working concentration (see Table 1) with serum-free cell culture medium.
3. Mix coating solution and transfer to the wells of tissue culture plate (see Table 2 for suggested volumes).
4. Make sure that the solution completely covers the bottom of the wells.
5. Incubate tissue culture plate at room temperature for an hour.
6. Aspirate coating solution and immediately plate cells. **Do not allow coated surface to dry out.**

Table 2: The suggested volumes of coating solution required per well:

| Plate type | Growth area (cm ²) | Volume of coating solution |
|------------|--------------------------------|----------------------------|
| 6-well | 9.5 | 1 – 1.5 ml |
| 12-well | 4 | 500 - 600 µl |
| 24-well | 2 | 250 - 300 µl |
| 48-well | 1 | 150 µl |
| 96-well | 0.3 – 0.6 | 50 µl |

Immunostaining of H9 hESCs cultured on Cultrex[®] Stem Cell Qualified Human Fibronectin, PathClear[®]

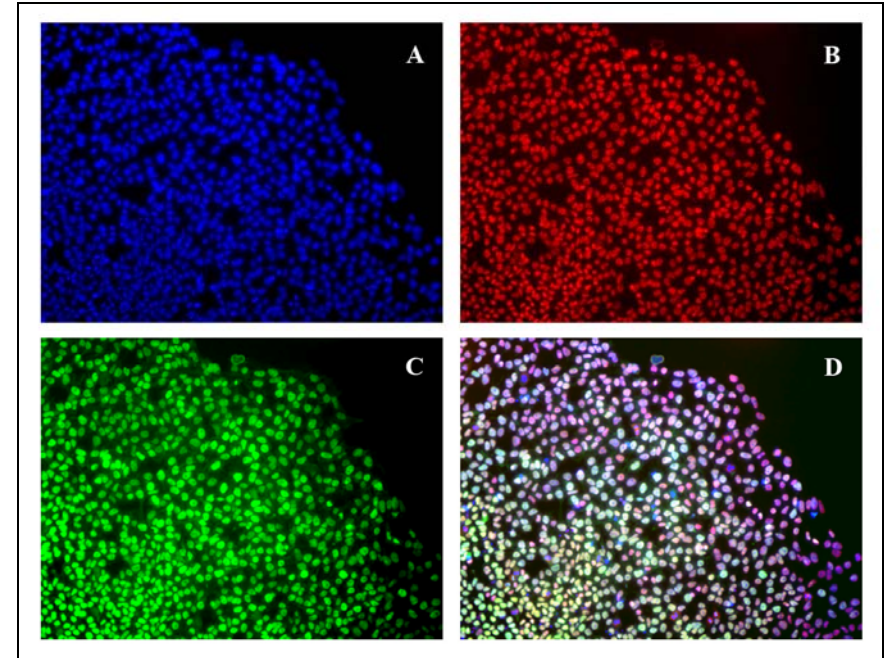


Fig.1. H9 human embryonic stem cells after three passages on Cultrex[®] Stem Cell Qualified Human Fibronectin, PathClear[®] maintain expression of the non-differentiated stem cell markers Sox 2 (B) and Oct-4 (C). Nuclear staining by DAPI shown on panel (A) and merged image shown on panel (D). Images courtesy of the Yanik lab, MIT <http://www.rle.mit.edu/bbng>

VI. References

1. Bilozur, M.E., and E.D. Hay. 1988. Neural crest cell migration in 3 dimensional matrix utilizes laminin, fibronectin or collagen. *Developments in Biologicals* **125**:19-33.
2. Arnaoutova, I., George, J., Kleinman, H.K., and G. Benton. 2011. Basement membrane matrix (BME) has multiple uses with stem cells. *Stem Cell Rev and Rep*, in press.
3. Angel, M., and M.F. Yanik. 2010. Innate immune suppression enables frequent transfection with RNA encoding reprogramming proteins. *PLoS ONE* **5**: e11756.

4. 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.
5. Vaheri A, Mosher DF. 1978. High molecular weight, cell surface-associated glycoprotein (fibronectin) lost in malignant transformation. *Biochim Biophys Acta*. **516**:1-25.
6. Mao Y, Schwarzbauer JE. 2005. Fibronectin fibrillogenesis, a cell-mediated matrix assembly process. *Matrix Biol* **24**:389-99.
7. Polyak K, Weinberg RA. 2009. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. *Nat Rev Cancer* **9**:265-73.
8. 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.
9. Hunt GC, Schwarzbauer JE. 2009. Tightening the connections between cadherins and fibronectin matrix. *Developmental Cell* **16**:327-8.
10. 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 α V β 5 integrin. *Stem Cell* **26**:2257-65.
11. Vuento M, Korkolainen M, Kuusela P, Holtta E. 1985. Isolation of a novel cell-attachment and spreading-promoting protein from human serum. *Biochem J*. **227**:421-7.
12. 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.
13. 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.
14. 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.
15. 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.
16. 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.
17. Kleinman H.K. 1999. The Laminins. *Proc. Indian Acad. Sci. (Chem.Sci.)*, **111**:179-184.
18. Xu C., Inokuma M.S., Denham J., Golds K., Kundu P., Gold, J. D., et al. 2001. Feeder-free growth of undifferentiated human embryonic stem cells. *Nature Biotechnology* **19**:971-5.
19. U.S. Patent 4,829,000

VII. Related products available from Trevigen.

| Catalog# | Description | Size |
|-------------|---|--------|
| 3415-001-03 | Cultrex® Stem Cell Qualified Human BME, PathClear® | 1 ml |
| 3420-001-03 | Cultrex® Stem Cell Qualified Human Fibronectin, PathClear® | 1 mg |
| 3421-001-03 | Cultrex® Stem Cell Qualified Human Vitronectin, PathClear® | 200 µg |
| 3400-010-03 | Cultrex® Stem Cell Qualified Laminin I, PathClear® | 1 mg |
| 3434-001-02 | Cultrex® Stem Cell Qualified BME, Growth Factor Reduced, PathClear® | 1 ml |
| 3434-005-02 | Cultrex® Stem Cell Qualified BME, Growth Factor Reduced, PathClear® | 5 ml |

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