CULTREX®

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

Catalog #: 3434-001-02 **Size:** 1 ml
3434-005-02 5 ml

Description: Cultrex Stem Cell Qualified **B**asement **M**embrane **E**xtract (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
Source: Murine Engelbreth-Holm-Swarm (EHS) tumor
Storage Buffer: Dulbecco's Modified Eagle's medium containing 10 µg/ml gentamycin 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 in a manual defrost freezer. **For optimal stability, store at –80°C in aliquots. Keep Frozen; repeated freeze-thaws will destroy product integrity.**

Material Qualification:

- 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.

*PathClear®: 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.

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TREVIGEN®

Sterility Testing:

- No bacterial or fungal growth detected after incubation at 37°C for 14 days following USP XXIV Chapter 71 sterility test.
- No mycoplasma contamination detected by PCR.
- Endotoxin concentrations ≤ 8 EU/ml by LAL assay.

Coating Procedures:

Refrigerator temperatures may vary; therefore thaw Cultrex® BME at 2-8°C overnight on ice in a refrigerator. BME gels in 15-30 minutes above 15°C; keeping the BME container and coated labware on ice will prevent gelling and extend working times. Bubbles may be prevented or eliminated from the BME by maintaining labware on ice during coating and centrifuging 300 x g for 10 minutes at 4°C.

There are many applications for Cultrex® BME, which require different thicknesses and concentrations. In general, BME, at a protein concentration ≥ 9 mg/ml, is used for differentiation studies of primary cells. Extract diluted below 9 mg/ml does not form a gel, and will only support the propagation of primary cells, but not their differentiation. For applications such as endothelial cell formation of capillary-like structures (Tube Assay), the differentiation of rat aorta tissue into capillary-like structures (Ring Assay), epithelial organoid formation, or tumor organoid formation, a thick gel is needed.

Thin Layer Method for Stem Cell Propagation (non-gelling)[13]:

1. Thaw BME as stated above.
2. Mix extract by slowly pipetting solution up and down; be careful not to introduce air bubbles.
3. Dilute the extract to desired concentration in cold serum-free medium. Empirical determination of the optimal coating concentration for your application may be required. A protein concentration of 150 µg/ml is a recommended starting concentration for the propagation of stem cells.
4. Add a sufficient amount of solution to cover the entire area onto growth surface. A volume of 300 µl per cm^2 is recommended.
5. Incubate coated object at room temperature for a minimum of 30 minutes or as long as 1 hour.
6. Aspirate coating solution and immediately plate cells. **Do not allow coated surface to dry out.**

Thick Gel Method:

1. Thaw BME as stated above.
2. Mix extract by slowly pipetting solution up and down; be careful not to introduce air bubbles.
3. Pipette 200-300 µl per cm^2 onto the growth surface.
4. Place coated object at 37 °C for 30 minutes.
5. Coated objects are ready for use.

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Immunostaining of H9 hESCs cultured on Cultrex® Stem Cell Qualified BME

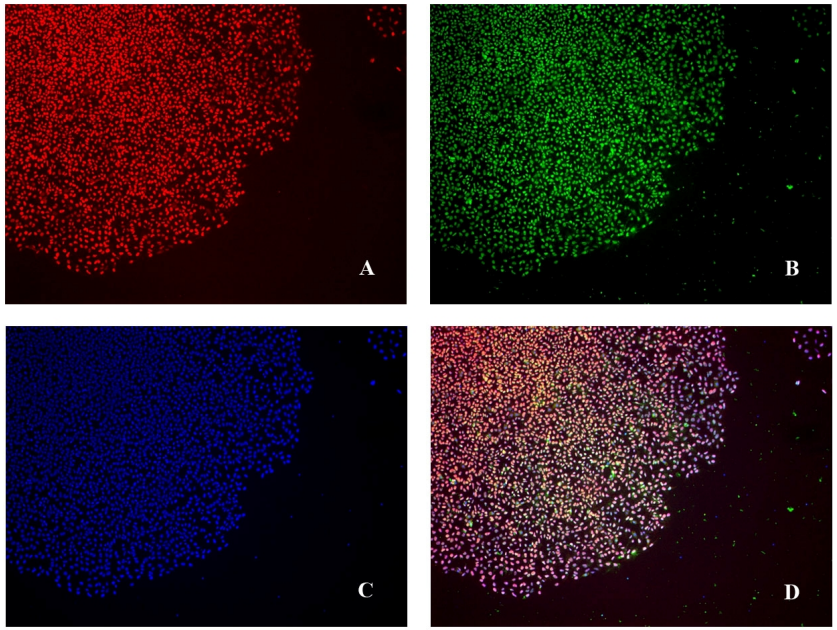

Fig. 1. H9 human embryonic stem cells after four passages on Cultrex Stem Cell Qualified BME maintain expression of the non-differentiated stem cell markers Oct-4 (A) and Nanog (B). Nuclear staining by DAPI shown on panel (C) and merged image of undifferentiation markers and DAPI shown on panel (D).
Images courtesy of the Yanik lab, MIT http://www.rle.mit.edu/bbng

Related Products:

Catalog#	Description	Size
3415-001-02	Cultrex® Human BME, PathClear®	1 ml
3430-005-02	Cultrex® BME with Phenol Red, PathClear®	5 ml
3431-005-02	Cultrex® BME with Phenol Red, Growth Factor Reduced, PathClear®	5 ml
3432-005-02	Cultrex® BME PathClear®	5 ml
3433-005-02	Cultrex BME no phenol red; reduced growth factor PathClear®	5 ml
3400-010-02	Cultrex® Mouse Laminin I, PathClear®	1 ml
3400-010-01	Cultrex® Mouse Laminin I	1 mg
3440-100-01	Cultrex® Rat Collagen I	100 mg
3410-010-01	Cultrex® Mouse Collagen IV	1 mg
3420-001-01	Cultrex® Human Fibronectin, PathClear®	1 mg
3416-001-01	Cultrex® Bovine Fibronectin, NZHD*	1 mg
3421-001-01	Cultrex® Human Vitronectin, PathClear®	50 µg
3417-001-01	Cultrex® Bovine Vitronectin, NZHD*	50 µg
3438-100-01	Cultrex® Poly-L-Lysine	100 ml
3439-100-01	Cultrex® Poly-D-Lysine	100 ml

*New Zealand Herd Derived

Catalog#	Description	Size
3445-048-01	Cultrex® 3-D Culture Matrix™ BME	15 ml
3446-005-01	Cultrex® 3-D Culture Matrix™ Laminin I	5 ml
3447-020-01	Cultrex® 3-D Culture Matrix™ Collagen I	100 mg
3430-005-01	Cultrex® BME with Phenol Red	5 ml
3432-005-01	Cultrex® BME without Phenol Red	5 ml
3431-005-01	Cultrex® BME with Phenol Red; Reduced Growth Factor	5 ml
3433-005-01	Cultrex® BME; no Phenol Red; Reduced Growth Factor	5 ml
3437-100-K	Cultrex® Cell Staining Kit	100 ml

References:

1. Albini, A., Y. Iwamoto, H. Kleinman, G. Martin, S. Aaronson, J. Kozlowski, and R. McEwan. 1987. A rapid *in vitro* assay for quantitating the invasive potential of tumor cells. Cancer Res. **47**:3239-3245.
2. Fridman, R., G. Giaccone, T. Kanemoto, G. Martin, A. Gazdar, and J. Mulshine. 1990. Reconstituted basement membrane (matrigel) and laminin can enhance the tumorigenicity and the drug resistance of small cell lung cancer cell lines. Proc. Natl. Acad. Sci. USA **87**:6698-6702.
3. Fridman, R., M. Kibbey, L.. Royce, M. Zain, T. Sweeney, D. Jicha, J. Yannelli, G. Martin, and H. Kleinman. 1991. Enhanced tumor growth of both primary and established human and murine tumor cells in athymic mice after coinjection with matrigel. J. Natl. Cancer Inst. **83**:769-774.
4. Fridman, R., T. Sweeney, M. Zain, G. Martin, and H. Kleinman. 1992. Malignant transformation of NIH-3T3 cells after subcutaneous co-injection with a reconstituted basement membrane (matrigel). Int. J. Cancer **51**:740-744.
5. Kubota, Y., H. Kleinman, G. Martin, and T. Lawley. 1988. Role of laminin and basement membrane proteins in the morphological differentiation of human endothelial cells in capillary-like structures. J. Cell Biol. **107**:1589-1598.
6. Ponce, M., M. Nomizu, M. Delgado, Y. Kuratomi, M. Hoffman, S. Powell, Y. Yamada, H. Kleinman, and K. Malinda. 1999. Identification of endothelial cell binding sites on the laminin γ1 chain. Circ. Res. **84**:688-694.
7. Salcedo, R., H. Young, M. Ponce, J. Ward, H. Kleinman, J. Murphy, and J. Oppenheim. 2001. Eotaxin (CCL11) induces *in vivo* angiogenic responses by human CCR3$^+$ endothelial cells. J. Immunol. **166**:7571-7578.
8. Eisenstein, M. 2006. Thinking outside the dish. Nature Methods **3**:1035-1043.
9. Benton, G., J. George, H.K. Kleinman, and I.P. Arnaoutova. 2009. Advancing Science and Technology Via 3D Culture on Basement Membrane Matrix. J. Cell. Physiol. **221**:18-25.
10. Arnaoutova, I., J. George, H.K. Kleinman, and G. Benton. 2009. The endothelial cell tube formation assay on basement membrane turns 20: state of the science and the art. Angiogenesis **12**:267-274.
11. U.S. Patent 4,829,000
12. U.S. Patent 5,158,874
13. Angel, M. and M. F. Yanik. 2010. Innate Immune Suppression Enables Frequent Transfection with RNA Encoding Reprogramming Proteins. *PLoS ONE*. **5**:e11756.

This product is made and marketed under patent license from the United States Public Health Service. Ref. U.S. Patent 4,829,000 issued May 9, 1989 and U.S. Patent 5,158,874 issued October 27, 1992, all entitled Reconstituted Membrane Complex with Biological Activity

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Protein Concentration:
Endotoxin (LAL):
Size:

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