CULTREX® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Cultrex® Stem Cell Qualified Reduced Growth Factor Basement Membrane Extract, PathClear®

Catalog #: 3434-005-02 **Size:** 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 embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) in an undifferentiated 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 healing. 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 without phenol red, with 10 µg/ml gentamicin sulfate.
Storage/Stability:	Product is stable for a minimum of two years from date of manufacture when stored at –80°C. See Certificate of Analysis for expiration date. **Avoid freeze-thaw cycles.**

Material Qualification:

Functional assay:

- Promotes the attachment of human iPSCs.
- Effectively maintains human iPSCs in a pluripotent state in a feeder-free culture.

Sterility testing:

- **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.
- No bacterial or fungal growth detected after incubation at 37°C for 14 days following USP sterility testing guidelines.
- Endotoxin concentration ≤ 8 EU/ml by LAL assay.

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Coating Procedures:

Thaw Cultrex® BME overnight at 2-8°C. Refrigerator temperatures may vary; therefore it is recommended to keep BME on ice in a refrigerator during thawing process. Thawed BME solidifies quickly at the temperatures above 15°C; when working with extract, keep it on ice to prevent untimely gelling.
There are many applications for Cultrex® BME, which require different thicknesses and concentrations. In general, BME at a protein concentration ≥ 10 mg/ml is used for differentiation studies of primary cells or stem cells. For applications such as endothelial cell formation of capillary-like structures (Tube Formation Assay), the differentiation of rat aorta tissue into capillary-like structures (Aortic Ring Assay), epithelial organoid formation, or tumor organoid formation, a thick gel is needed.
Some applications, such as propagation of hESCs and iPSCs in feeder-free culture, require a thin layer coating and not a thick gel; therefore, the thin layer method should be used.

Thin Layer Method for Stem Cell Propagation in Feeder-free Culture (non-gelling):

1. Thaw BME as stated above.
2. Mix BME by slowly pipetting solution up and down; be careful not to introduce air bubbles.
3. Dilute BME 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 an hour.
6. Aspirate coating solution and immediately plate cells. **Do not allow coated surface dry out.**

NOTE: The coated plates can be prepared in advance:

7. Follow steps 1 to 4; then seal the plates with Parafilm® and store for up to two weeks in a refrigerator at 2-8°C.
8. Incubate coated plates at room temperature for an hour.
9. Continue with step 6.

Thick Gel Method:

1. Thaw BME as stated above.
2. Mix BME 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.

References:

1. Bilozur, M.E., and E.D. Hay. 1988. Neural crest cell migration in 3 dimentional matrix utilizes laminin, fibronectin or collagen. *Developments in Biologicals* **125**:19-33.
2. Amit M, Shariki C, Margulets V, Itskovitz-Eldor J. 2004. Feeder layer- and serum-free culture of human embryonic stem cells. *Biol Reprod*. Mar; **70**(3): 837-45.
3. Levenstein ME, Ludwig TE, Xu RH, Llanas RA, VanDenHeuvel-Kramer K, Manning D, Thomson JA. 2005. Basic fibroblast growth factor support of human embryonic stem cell self-renewal. *Stem Cells*. Mar; **24**(3):568-74.
4. Ludwig TE, Bergendahl V, Levenstein ME, Yu J, Probasco MD, Thomson JA. 2006. Feeder-independent culture of human embryonic stem cells. *Nat Methods*. Aug;**3**(8): 637-46.
5. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. 2007. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. Dec **21**;318(5858): 1917-20.
6. Angel, M., and M.F. Yanik. 2010. Innate immune suppression enables frequent transfection with RNA encoding reprogramming proteins. *PLoS ONE* **5**: e11756.
7. Arnaoutova I, George J, Kleinman HK, Benton G. 2012. Basement membrane matrix (BME) has multiple uses with stem cells. *Stem Cell Rev*. Mar; **8**(1); 163-9.

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

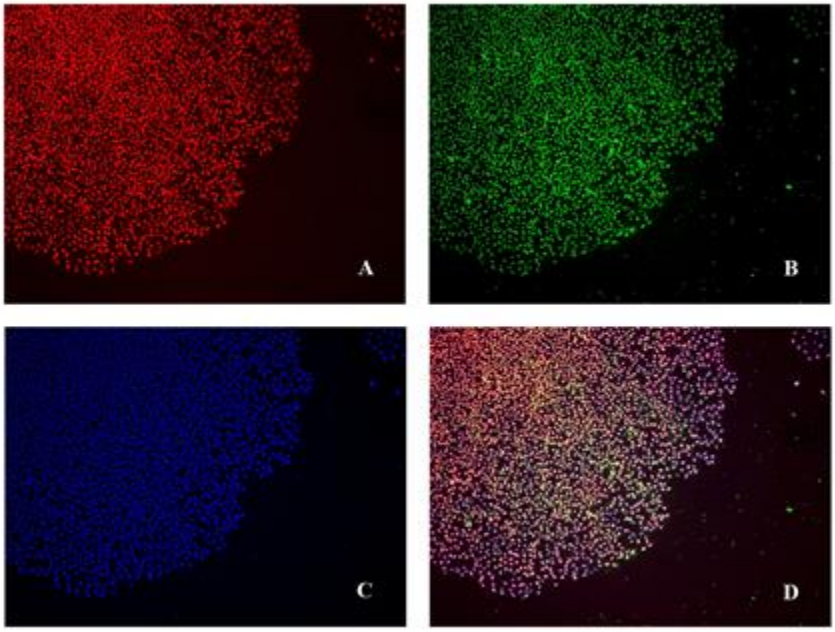

Figure 1. H9 human embryonic stem cells after four passages on Cultrex® Stem Cell Qualified BME, maintain expression of pluripotency markers Oct-4 (A) and Nanog (B). Nuclear staining by DAPI shown on panel (C) and merged image of Oct-4, Nanog and DAPI shown on panel (D).
Images courtesy of the Yanik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

Related Products:

Catalog#	Description	Size
3415-001-03	Cultrex® Stem Cell Qualified Human BME, PathClear®	1 mg
3400-010-03	Cultrex® Stem Cell Qualified Laminin I, PathClear®	1 mg
3420-001-03	Cultrex® Stem Cell Qualified Human Fibronectin, PathClear®	1 mg
3420-001-03	Cultrex® Stem Cell Qualified Human Vitronectin, PathClear®	200 µg
3432-005-01	Cultrex® Basement Membrane Extract, PathClear®	5 ml
3433-005-01	Cultrex® Reduced Growth Factor BME, PathClear®	5 ml
3532-005-02	Cultrex® Basement Membrane Extract, Type 2, PathClear®	5 ml
3533-005-02	Cultrex® RGF BME, Type 2, PathClear®	5 ml
3632-005-02	Cultrex® Basement Membrane Extract, Type 3, PathClear®	5 ml
3410-010-01	Cultrex® Mouse Collagen IV	1 mg
3440-100-01	Cultrex® Rat Collagen I	100 mg
3442-050-01	Cultrex® Bovine Collagen I	50 mg
3445-005-01	Cultrex® 3-D Culture Matrix™ BME, PathClear®	5 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
3438-100-01	Cultrex® Poly-L-Lysine	100 ml
3439-100-01	Cultrex® Ploy-D-Lysine	100 ml

Related Assays and Kits:

Catalog#	Description	Size
3500-096-K	Cultrex® 3D Spheroid Cell Invasion Assay	96 samples
3510-096-K	Cultrex® 3D Spheroid Fluorometric Proliferation/Viability Assay	96 samples
3511-096-K	Cultrex® 3D Spheroid Colorimetric Proliferation/Viability Assay	96 samples
3434-SCQ-K	Cultrex® Stem Cell Qualified Protein Set	1 kit
3445-096-K	Cultrex® 3-D Culture BME Cell Proliferation Kit	96 samples
3448-020-K	Cultrex® 3-D Culture Cell Harvesting Kit	20 samples

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Storage: -80˚C

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