

CULTREX[®] Product Data

For Research Use Only. Not For Use in Diagnostic Procedures

Cultrex[®] Reduced Growth Factor Basement Membrane Extract, Type R1, PathClear[®]

Catalog #: 3433-001-R1

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 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[®] Basement Membrane Extract (BME) is a soluble form of basement membrane purified from Engelbreth-Holm-Swarm (EHS) tumor. The extract gels at 37°C to form a reconstituted basement membrane. The major components of BME include laminin, collagen IV, entactin and heparin sulfate proteoglycan.

BME can be used in a multiple applications, under a variety of cell culture conditions, for maintaining growth or promoting differentiation of primary endothelial, epithelial, smooth muscle and stem cells. BME can also be utilized in cell attachment, neurite outgrowth, angiogenesis, *in vitro* cell invasion and *in vivo* tumorigenicity assays.

Recently we have developed an additional formulation of Cultrex[®] BME known as **Cultrex[®] BME Type R1**. **Cultrex[®] BME Type R1** provides a proprietary formulation that has higher tensile strength when compared to our other products Cultrex[®] BME, Cultrex[®] BME Type 2 and Cultrex[®] BME Type 3. Our new **Cultrex[®] BME Type R1** has a higher concentration of entactin, one of the BME components that connects laminins and collagens reinforcing the hydrogel structure. **Cultrex[®] BME Type R1** has been specifically designed to culture tissue organoids.

Specifications:

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 3 months from date of shipment when stored at -20°C in a manual defrost freezer.
For optimal stability, store at -80°C. Avoid freeze-thaw cycles.

Material Qualification:

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.

Material Qualification (Continued):

Functional assays:

- BME type R1 supports culture of human gastric or small intestine organoids.
- Tube formation assay - BME type R1 promotes formation of capillary-like structures by human (HBMVEC; HUVEC) or mouse (SVEC4-10) endothelial cells.

Gelling assay:

- BME type R1 gels in less than 30 minutes at 37°C, and maintains the gelled form in culture medium for a minimum of 14 days at 37°C.

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. 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 primary cells, require a thin layer coating and not a thick gel; therefore, the thin layer method should be used.

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 μ l per cm^2 onto the growth surface.
4. Place coated object at 37°C for 30 minutes.
5. Coated objects are ready for use.

Thin Layer Method (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 μ g/ml is a recommended starting concentration for the propagation of primary cells.
4. Add a sufficient amount of solution to cover the entire area onto growth surface. A volume of 300 μ l 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.**

References:

1. 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.
2. Arnaoutova IP and Kleinman HK. 2010. In vitro angiogenesis: endothelial cell tube formation on gelled basement membrane extract. *Nature Protocol.* **5** (4): 628-35.
3. Benton G, Kleinman HK, George J, Arnaoutova I. 2011. Multiple uses of basement membrane matrix (BME/Matrigel) in vitro and in vivo with tumor cells. *Int. J. Cancer.* **128** (8); 1751-7.
4. Fridman R, Benton G., Arnaoutova I, Kleinman HK, Bonfil RD. 2012. Increased initiation and growth of tumor cell lines, cancer stem cells and biopsy material in mice using basement membrane matrix protein (Cultrex or Matrigel) co-injection. *Nature Protocol May* **17**:7 (6); 1138-44.
5. Barker, N., et al., *Lgr5+ve Stem Cells Drive Self-Renewal in the Stomach and Build Long-Lived Gastric Units In Vitro.* *Cell Stem Cell*, 2010. **6**(1): p. 25-36.
6. Sato, T., et al., *Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche.* *Nature*, 2009. **459**(7244): p. 262-265.

7. Sato, T. and H. Clevers, *Growing Self-Organizing Mini-Guts from a Single Intestinal Stem Cell: Mechanism and Applications*. Science, 2013. **340**(6137): p. 1190-1194.
8. Jung, P., et al., *Isolation and in vitro expansion of human colonic stem cells*. Nat Med, 2011. **17**(10): p. 1225-7.

Cultrex BME Selection Chart			
NAME	BUFFER	CONCENTRATION	RECOMMENDED APPLICATIONS
Cultrex® Original BME	DMEM	12-18 mg/ml	General (2D Culture, 3-D Culture, Attachment, Invasion, etc.)
Cultrex® Stem Cell Qualified BME	DMEM	12-18 mg/ml	Attachment and Maintenance of Stem Cells
Cultrex® 3-D Culture Matrix™ BME	DMEM	14-16 mg/ml	3-D Culture
Cultrex® Organoid Qualified BME, Type 2	DMEM	12-18 mg/ml	Robust Organoid Culture
Cultrex® BME, Type 3	RPMI1640	12-18 mg/ml	Xenograft/Tumorgraft
Cultrex® Organoid Qualified BME, Type R1	DMEM	12-18 mg/ml	Difficult to Grow Organoid Culture

Related Products:

Catalog#	Description	Size
3432-005-01	Cultrex® Basement Membrane Extract, PathClear®	5 ml
3433-005-01	Cultrex® Reduced Growth Factor BME, PathClear®	5 ml
3533-005-02	Cultrex® Reduced Growth Factor BME, Type 2, PathClear®	5 ml
3632-005-02	Cultrex® Basement Membrane Extract, Type 3, PathClear®	5 ml
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
3434-005-02	Cultrex® Stem Cell Qualified RGF BME, PathClear®	5 ml
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
3400-010-01	Cultrex® Mouse Laminin I	1 mg
3400-010-02	Cultrex® Mouse Laminin I, PathClear®	1 mg
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
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
3710-001-01	Cultrex® HA-R-Spondin1-Fc 293T Cells	1x10 ⁶ cells

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
3470-096-K	Cultrex® In Vitro Angiogenesis Assay, Tube Formation Kit	96 samples
3471-096-K	Cultrex® In Vitro Angiogenesis Assay, Endothelial Cell Invasion Kit	96 samples
3450-048-SK	Cultrex® Directed In Vivo Angiogenesis Assay (DIVAA™) Starter Kit	48 samples
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



**RGF BME Type R1
PathClear®**

Cat#: 3433-005-R1
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