CULTREX® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Cultrex® Basement Membrane Extract, Type 3, PathClear®

Catalog #:	3632-001-02	**Size:**	1 ml
	3632-005-02		1 ml
	3632-010-02		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® **B**asement **M**embrane **E**xtract (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 two additional formulations of Cultrex® BME known as Cultrex® BME Type 2 and **Cultrex® BME Type 3**. Cultrex® BME Type 2 provides a proprietary formulation that is higher in tensile strength when compared to our original BME, while **Cultrex® BME Type 3** is physiologically aligned with the *in vivo* solid tumors environment and is recommended for xenografts and other *in vivo* applications.

Specifications:

Concentration:	12 - 18 mg/ml.
Source:	Murine Engelbreth-Holm-Swarm (EHS) tumor.
Storage buffer:	RPMI1640 medium without 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. 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.

Functional assay:

- Tumor Growth Assay - BME Type 3 supports proliferation and growth of breast cancer cells (MCF7) embedded in the matrix for minimum of 8 days.

Gelling assay:

- BME 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.

Cultrex® BME Selection Chart

NAME	BUFFER	TENSILE STRENGTH	CONCENTRATION	APPLICATIONS
Cultrex® BME, PathClear®	DMEM	MEDIUM	12-18 mg/ml	xenograft/tumorgraft 2D cell culture 3D culture spheroids/organoids stem cell
Cultrex® BME, Type 2, PathClear®	DMEM	HIGH	12-18 mg/ml	xenograft/tumorgraft 2D cell culture 3D culture spheroids/organoids stem cell
Cultrex® BME, Type 3, PathClear®	RPMI1640	HIGH	12-18 mg/ml	xenograft/tumorgraft

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 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.

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 µg/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 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.**

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. Eisenstein, M. 2006. Thinking outside the dish. Nature Methods **3**:1035-1043.
8. 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.
9. 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**(3); 267-74.
10. Arnaoutova IP and Kleinman HK. 2010. In vitro angiogenesis: endothelial cell tube formation on gelled basement membrane extract. Nature Protocol. **5** (4); 628-35.
11. 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.
12. 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.
13. 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.

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
3532-005-02	Cultrex® Basement Membrane Extract, Type 2, PathClear®	5 ml
3533-005-02	Cultrex® Reduced Growth Factor BME, Type 2, 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

***New Zealand Herd Derived**

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
3450-048-K	Cultrex® DIVAA™ Kit	48 samples
3450-048-IK	Cultrex® DIVAA™ Inhibition Kit	48 samples
3465-024-K	Cultrex® 24 well Migration Cell Assay	24 inserts
3455-024-K	Cultrex® 24 well BME Cell Invasion Assay	24 inserts
3456-024-K	Cultrex® 24 well Laminin I Cell Invasion Assay	24 inserts
3457-024-K	Cultrex® 24 well Collagen I Cell Invasion Assay	24 inserts
3458-024-K	Cultrex® 24 well Collagen IV Cell Invasion Assay	24 inserts
3465-096-K	Cultrex® 96 well Migration Cell Assay	96 samples
3455-096-K	Cultrex® 96 well BME Cell Invasion Assay	96 samples
3456-096-K	Cultrex® 96 well Laminin I Cell Invasion Assay	96 samples
3457-096-K	Cultrex® 96 well Collagen I Cell Invasion Assay	96 samples
3458-096-K	Cultrex® 96 well Collagen IV Cell Invasion Assay	96 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

BME Type 3
PathClear®
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(Manual Defrost)
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