CULTREX® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Cultrex® BME with Phenol Red*
**PathClear®

Catalog #: 3430-005-02 (3432-005-02 + 3430-50-01)* **Size:** 5 ml
3430-001-02 (3432-001-02 + 3430-50-01)* 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® **B**asement **M**embrane **E**xtract (BME) is a soluble form of basement membrane purified from Engelbreth-Holm-Swarm (EHS) tumors. 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 for growth promotion or for the differentiation of precursor cells, under a variety of cell culture conditions, involving primary epithelial, endothelial and smooth muscle cells. BME has also been employed in cell attachment, neurite outgrowth, angiogenesis, *in vitro* cell invasion and *in vivo* tumorigenicity assays.

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 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. Keep Frozen; repeated freeze-thaws will destroy product integrity.**

Material Qualification:

Gelling: 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.

Functional Assay:

- Tube Assay: BME promotes formation of capillary-like structures by human (HBMVEC; HUVEC) and mouse (SVEC4-10) endothelial cells.

* Phenol red (cat# 3430-50-01), provided separately at 100X, needs to be added at 1:100 (v/v) prior to use.

**PathClear®: Negative by PCR test for: mycoplasma, 17 bacterial and virus strains typically included in mouse antibody production (MAP) testing including LDEV, plus 13 additional murine infectious agents, for a total of 31 organisms and viruses.

 E4/28/10v1

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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 ≤ 20 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 5-10 minutes above 15 °C; therefore it is **unnecessary** to keep it on ice if used within 5 minutes and the environmental temperature does not exceed 15 °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) a thin gel is needed. For applications such as the differentiation of rat aorta tissue into capillary-like structures (Ring Assay), or cell invasion assays, a thick gel is needed. Some applications, such as propagation of primary cells, only need a protein layer and not a protein matrix; therefore, the layer method should be used.

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 150-200 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 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 50 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. Place coated object at 37 °C, and 5% CO_2 for a minimum of 2 hours or as long as overnight.
6. Aspirate medium.
7. Coated objects are ready for use.

Related Products:

Catalog#	Description	Size
3455-024-K	Cultrex® 24 Well BME Cell Invasion Assay	24 inserts
3480-024-K	CultreCoat® 24 Well BME-Coated 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
3455-096-K	Cultrex® 96 well BME Cell Invasion Assay	96 samples

Catalog#	Description	Size
3465-024-K	Cultrex® 24 well Migration Cell Assay	96 samples
3465-096-K	Cultrex® 96 well Migration Cell Assay	96 samples
3456-096-K	Cultrex® Laminin I Cell Invasion Assay	96 samples
3457-096-K	Cultrex® Collagen I Cell Invasion Assay	96 samples
3458-096-K	Cultrex® Collagen IV Cell Invasion Assay	96 samples
3490-096-K	CultreCoat® BME 96 Well Cell Adhesion Assay	96 samples
3496-096-K	CultreCoat® 96 Well Adhesion Protein Array	96 samples
3450-048-SK	Cultrex® Directed In Vivo Angiogenesis Assay (DIVAA™) Starter	48 samples
3450-048-K	Cultrex® DIVAA™ Kit	48 samples
3450-048-IK	Cultrex® DIVAA™ Inhibition Kit	48 samples

Accessories:

Catalog#	Description	Size
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
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
3415-001-02	Cultrex® Human BME, PathClear®	1 ml
3431-005-02	Cultrex® BME with Phenol Red, Reduced Growth Factor 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
3437-100-K	Cultrex® Cell Staining Kit	100 ml
3450-048-05	CellSperse™	15 ml

*New Zealand Herd Derived

References:

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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 doi:10.1007/s10456-009-9146-4
11. U.S. Patent 4,829,000
12. U.S. Patent 5,158,874

Lot Specific Data:

Protein Concentration:
Endotoxin (LAL):
Size:

Cultrex®

BME with Phenol Red
PathClear®
Cat#: 3430-005-02
Storage: ≤ -20 °C
(Manual Defrost)
1-800-873-8443