For Research Use Only. Not For Use In Humans.

Cultrex® Basement Membrane Extract Reduced Growth Factor (phenol red free)

Catalog #:	3433-005-01	**Size:** 5 mL	**Concentration:** 12 - 18 mg/mL
	3433-010-01	2 x 5 mL	
	3433-050-01	10 x 5 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 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 the BME include laminin, collagen IV, entactin, and heparin sulfate proteoglycan. The BME can be used for promotion and maintenance of a differentiated phenotype in a variety of cell cultures including primary epithelial cells, endothelial cells, and smooth muscle cells. It has been employed in angiogenesis assays, tumor cell invasion assays, and as a vehicle to augment the tumorigenicity of injected tumor cells in nude mice.

The reduction in specific growth factor concentrations allows use of the BME (Reduced Growth Factor) to examine the effects of specific growth factors in applications requiring BME.

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 Conditions: Product is stable for at least 3 months from the date of receipt when stored at ≤ -20° C or at ≤ -80° C in a manual defrost freezer. For optimal stabilty, store at ≤ -80° C. Keep frozen; Avoid repeated freeze-thaw cycles.

Specifications:
Gelling: Basement Membrane Extract 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.

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Functional Assays:
- Tube Assay: Basement Membrane Extract promotes differentiation of a mouse endothelial cell line derived from axillary lymph node (SVEC4-10) into capillary-like structures.
- Ring Assay: Basement Membrane Extract promotes differentiation of mouse aorta tissue to form capillary-like structures.

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: Thaw extract at 2 - 8° C overnight. Refrigerator temperatures may vary; therefore, thaw extract on ice in a refrigerator. Cultrex Basement Membrane Extract 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 25° C. It is also unnecessary to prechill pipette tips, tubes, plates, or any other object that may come in contact with the extract.

There are many applications for Cultrex Basement Membrane Extract, which require different thicknesses and concentrations. In general, Basement Membrane Extract, at a protein concentration ≥ 9 mg/mL, is used for differentiation studies of primary cells. Diluted extract (protein concentration ≤ 9 mg/mL) will only support the propagation of primary cells, but not their differentiation. For applications such as endothelial cell differentiation into capillary-like structures (Tube Assay) a thin gel is needed. For applications such as the differentiation of mouse 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.

Thin Gel Method:
1. Thaw Cultrex Basement Membrane Extract as stated above.
2. Mix extract by slowly pipetting solution up and down. Be careful not to introduce air bubbles.
3. Place 50 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.

Thick Gel Method:
1. Thaw Cultrex Basement Membrane Extract as stated above.
2. Mix extract by slowly pipetting solution up and down. Be careful not to introduce air bubbles.
3. Place 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 Cultrex Basement Membrane Extract 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 0.1 mg/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.
5. Place coated object at 37° C for 60 minutes or until dry.
6. Coated objects are ready for use.

References:

1. Albini, A. *et al.* (1987) *A rapid in vitro assay for quantitating the invasive potential of tumor cells.* Cancer Research **47**:3239.
2. Fridman, R. *et al.* (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.
3. Fridman, R. *et al.* (1991) *Enhanced tumor growth of both primary and established human and murine tumor cells in athymic mice after co-injection with matrigel.* J. Natl. Cancer Inst. **83**:769.
4. Fridman, R. *et al.* (1992) *Malignant transformation of NIH-3T3 cells after subcutaneous co-injection with a reconstituted basement membrane (matrigel).* Int. J. Cancer **51**:740.
5. Kubota, Y. *et al.* (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.
6. Ponce, M. *et al.* (1999) *Identification of endothelial cell binding sites on the laminin g1 chain.* Circ. Res. **84**:688.
7. Salcedo, R. *et al.* (2001) *Eotaxin (CCL11) induces in vivo angiogenic responses by human CCR3+ endothelial cells.* J. Immun. **166**:7571.
8. U.S. Patent 4,829,000.
9. U.S. Patent 5,158,874.

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Storage: -20° C

R&D Systems

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