For Research Use Only. Not For Use In Humans.

Cultrex® Basement Membrane Extract without Phenol Red

Catalog #: 3432-005-01 **Size:** 5 mL
3432-010-01 2 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 BME include laminin, collagen IV, entactin, and heparin sulfate proteoglycan. 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, neurite outgrowth assays, and tumor cell invasion assays.

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 no phenol red.

Storage Conditions: Product is stable for a minimum of 3 months from the 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 Qualifications:
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.

Functional Assays:
- Tube Assay: BME promotes formation of capillary-like structures by human (HBMVEC; HUVEC) and mouse (SVEC4-10) endothelial cells.
- Ring Assay: BME promotes sprouting of capillary-like structures from rat aorta tissue.

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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 differentiation into 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.

Thin 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. 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 BME 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 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 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) Cancer Res. **47**:3239.
2. Fridman, R. *et al.* (1990) Proc. Natl. Acad. Sci. USA **87**:6698.
3. Fridman, R. *et al.* (1991) J. Natl. Cancer Inst. **83**:769.
4. Fridman, R. *et al.* (1992) Int. J. Cancer **51**:740.
5. Kubota, Y. *et al.* (1988) J. Cell Biol. **107**:1589.
6. Ponce, M. *et al.* (1999) Circ. Res. **84**:688.
7. Salcedo, R. *et al.* (2001) J. Immun. **166**:7571.

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