DATENBLATT

Cultrex® BME with phenol red, reduced growth factor, PathClear®

Cat. # 3431-005-02

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, proli-feration, 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 growth promotion or for the differentiation of precursor cells, under a variety of cell culture conditions, involving stem, 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 in aliquots. Keep Frozen; repeated freeze-thaws will destroy product integrity.

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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, plus 13 additional murine infectious agentsincluding LDEV, for a total of 31 organisms and viruses.

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 15-30 minutes above 15 °C; keeping the BME container and coated labware on ice will prevent gelling and extend working times. Bubbles may be prevented or eliminated from the BME by maintaining labware on ice during coating and centrifuging 300 x g for 10 minutes at 4 °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), the differentiation of rat aorta tissue into capillary-like structures (Ring Assay), epithelial organoid formation, or tumor organoid formation, 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 thin 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 cm2 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.

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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 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. A volume of 300 ml per cm2 is recommended.
5. Place coated object at 37 °C, and 5% CO2 for a minimum of 2 hours or as long as overnight.
6. Aspirate medium.
7. Coated objects are ready for use.

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