

PRODUCT 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 Basement Membrane Extract (BME) is a soluble form of basement membrane purified from Engelbreth-Holm-Swarm (EHS) tumor. Cultrex BME gels at 37 °C to form a reconstituted basement membrane. The major components of BME include laminin, collagen IV, entactin, and heparin sulfate proteoglycan.

Cultrex BME can be used in multiple applications, including maintaining growth or promoting differentiation of primary endothelial, epithelial, smooth muscle, stem cells, and organoid/3-D cell cultures. It can also be utilized in cell attachment, neurite outgrowth, angiogenesis, *in vitro* cell invasion, and *in vivo* tumorigenicity assays.

INTENDED USE

Cultrex Reduced Growth Factor Basement Membrane Extract (RGF BME), Type R1 provides a proprietary formulation that is specifically designed to culture tissue organoids. Cultrex RGF BME, Type R1 has a higher tensile strength compared to other BME derivatives due to a higher concentration of entactin, one of the BME components that connects laminins and collagens to reinforce the hydrogel structure.

PRODUCT SPECIFICATIONS

| | |
|-----------------------|---|
| Concentration | 8-12 mg/mL as determined by Lowry assay. |
| Source | Murine Engelbreth-Holm-Swarm (EHS) tumor. |
| Storage Buffer | Dulbecco's Modified Eagle's Medium without phenol red, containing 10 µg/mL gentamicin sulfate. |
| Stability | Product is stable for two years from date of manufacture. See lot specific Certificate of Analysis for expiration date. |
| Storage | Store at ≤ -70 °C. Product may be thawed and dispensed into working aliquots. Avoid freeze-thaw cycles. |

PRECAUTION

When handling biohazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- Results may vary due to variations among tissue/cells derived from different donors or sources.

MATERIAL QUALIFICATIONS

Sterility Testing:

- PathClear - Tested negative by PCR test for 31 organisms and viruses, including: mycoplasma, 17 bacterial and virus strains typically included in mouse antibody production (MAP) testing, and 13 additional murine infectious agents including LDEV.
- Tested following USP <71> sterility guidelines.
- Endotoxin concentration ≤ 8 EU/mL by LAL assay.

Functional Assays:

- Organoid culture – Cultrex RGF BME, Type R1 supports culture of human gastric or small intestine organoids.
- Tube formation assay – Cultrex RGF BME, Type R1 promotes formation of capillary-like structures by human (HBMVEC; HUVEC) or mouse (SVEC4-10) endothelial cells.

Gelling Assay:

- Cultrex RGF BME, Type R1 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.

COATING PROCEDURES:

Thaw Cultrex RGF BME, Type R1 overnight at 2-8 °C. Refrigerator temperatures may vary, therefore it is recommended to keep BME on ice in a refrigerator during the thawing process. Thawed BME solidifies quickly at temperatures above 15 °C; when working with BME, keep it on ice to prevent untimely gelling.

There are many applications for Cultrex RGF BME, Type R1 which require different thicknesses and concentrations. A thick gel is needed 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. 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 Cultrex BME as stated above.
2. Mix Cultrex 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 Cultrex BME as stated above.
2. Mix Cultrex BME by slowly pipetting solution up and down; be careful not to introduce air bubbles.
3. Dilute Cultrex BME to desired concentration in **cold** serum-free medium. A 1:100 dilution is recommended for the propagation of primary cells. Empirical determination of the optimal coating concentration for your application may be required.
4. Add a sufficient amount of solution to cover the entire growth surface area. A volume of 300 μL per cm^2 is recommended.
5. Incubate coated object at room temperature for one hour.
6. Aspirate coating solution and immediately plate cells. **Do not allow coated surface to dry out.**

REFERENCES

1. Benton, G. *et al.* (2009) *J. Cell. Physiol.* **221**:18.
2. Arnaoutova, I.P. and H. K. Kleinman (2010) *Nature Protocols* **5**:628.
3. Benton, G. *et al.* (2011) *Int. J. Cancer* **128**:1751.
4. Fridman, R. *et al.* (2012) *Nature Protocols* **7**:1138.
5. Barker, N. *et al.* (2010) *Cell Stem Cell* **6**:25.
6. Sato, T. *et al.* (2009) *Nature* **459**:262.
7. Sato, T. and H. Clevers (2013) *Science* **340**:1190.
8. Jung, P. *et al.* (2011) *Nature Med.* **17**:1225.