

<b>Catalog Number:</b>	<b>Size:</b>
BME001-01	1.0 mL
BME001-05	5.0 mL
BME001-10	2x5.0 mL

## 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. The major components of the basement membrane include laminin, collagen IV, entactin/nidogen, and heparan sulfate proteoglycan. 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.

Cultrex UltiMatrix Reduced Growth Factor (RGF) Basement Membrane Extract (BME) is a soluble form of basement membrane that provides high tensile strength, enhanced levels of entactin/nidogen, elevated protein concentration, and robust clarity and purity. Purified from mouse Engelbreth-Holm-Swarm (EHS) tumor, Cultrex UltiMatrix RGF BME is viscous at 2-8 °C and gels at 37 °C to form a reconstituted basement membrane. Employing an optimized manufacturing process that maximizes purification and solubilization of extracellular matrix protein, Cultrex UltiMatrix RGF BME is a cell scaffolding hydrogel with substantial compositional enhancements and performance benefits for 2D and 3D cell culture applications.

## INTENDED USE

Cultrex UltiMatrix RGF BME is an optimized and effective growth scaffold for organoid, pluripotent stem cell, and advanced 3-D culture applications. It can be used undiluted or diluted to a specific protein concentration. Detailed instructions for use are provided in the Protocols section.

## PRODUCT SPECIFICATIONS

<b>Concentration</b>	10-12 mg/mL as determined by Lowry assay.
<b>Endotoxin</b>	Endotoxin concentration $\leq$ 7 EU/mL by LAL assay.
<b>Source</b>	Murine Engelbreth-Holm-Swarm (EHS) tumor.
<b>Storage Buffer</b>	Dulbecco's Modified Eagle's Medium without phenol red, containing 50 µg/mL gentamicin sulfate.
<b>Stability</b>	Product is stable for three years from date of manufacture when stored at $\leq$ -70 °C. See lot specific Certificate of Analysis for expiration date.
<b>Storage</b>	Store at $\leq$ -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 be variable across tissue/cells derived from different donors or sources.

## MATERIAL QUALIFICATIONS

### Sterility Testing:

- Tested following USP <71> sterility guidelines.
- PathClear - Tested negative by PCR test for 32 organisms and viruses, including: mycoplasma, 20 virus strains typically included in mouse antibody production (MAP) testing including LDEV, and 12 additional murine bacterial and viral infectious agents.

### Functional Assays:

- Organoid culture – Supports culture of human small intestine or colon organoids. Supports mouse intestinal organoids.
- Pluripotent stem cell culture – Promotes the attachment of human induced pluripotent stem cells (iPSCs) and effectively maintains them in a pluripotent state under a feeder-free conditions.
- Protein composition - Presence of high levels of entactin by SDS-PAGE and Coomassie staining.

### Gelling Assays:

- Dome formation - Supports dome formation at a concentration of 8 mg/mL and maintains dome structure for 7 days.
- Gel formations - Gels in less than 30 minutes at 37 °C and maintains the gelled form in culture medium for a minimum of 12 days at 37 °C.

## PROTOCOLS

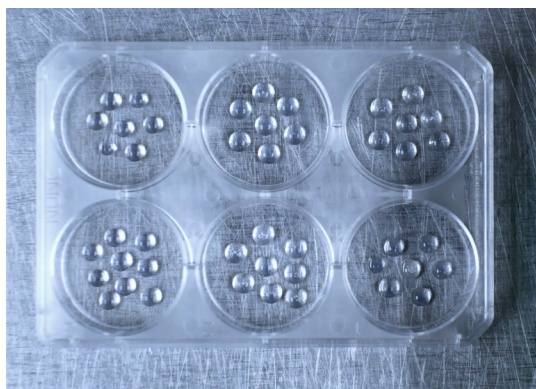
### Thawing Cultrex UltiMatrix BME

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

### Dome Formation for Organoid Culture

Cultrex UltiMatrix RGF BME is designed to support organoid culture due to its high tensile strength, elevated protein concentration, and optimized levels of entactin. It can be used undiluted or diluted to a specific protein concentration using ice-cold cell culture medium. The following protocol describes a basic protocol for use.

1. Thaw Cultrex UltiMatrix RGF BME as stated above.
2. Mix Cultrex UltiMatrix RGF BME by slowly pipetting solution up and down; be careful not to introduce air bubbles.  
**Note:** *If desired, dilute Cultrex UltiMatrix BME in ice-cold cell culture media to optimize the protein concentration empirically determined for your specific application. For dome formation, we recommend a final Cultrex UltiMatrix RGF BME concentration of > 7 mg/mL.*
3. Prepare organoids, adult stem cells, or pluripotent stem cell starting population by centrifuging cells at 500 x g for 3 minutes. Aspirate medium.
4. Resuspend pellet in Cultrex UltiMatrix RGF BME. Pipette up and down to resuspend starting material in Cultrex UltiMatrix RGF BME; be careful not to introduce air bubbles.  
**Note:** *The optimal density of cells/mL of Cultrex UltiMatrix BME may vary by organoid or by the starting cell population and must be empirically determined by application. For example, we recommend intestinal organoids be resuspended as 10,000 organoids/mL.*
5. Using a pre-warmed (37 °C) tissue culture plate, dispense 50 µL of the Cultrex UltiMatrix RGF BME/organoid suspension into the center of the well of culture plate (Figure 1).  
**Note:** *The Cultrex UltiMatrix RGF BME/organoid suspension should be dispensed slowly to promote dome formation. Cultrex BME-contained organoids should not touch the sides of the well. If necessary, use reverse pipetting to dispense BME by aspirating to the second stop of commonly used micropipettes and dispensing to the first stop.*



**Figure 1. Cultrex UltiMatrix RGF BME Domes.** Cultrex UltiMatrix BME was diluted to 8 mg/mL using ice-cold culture medium and dispensed as 50  $\mu$ L domes.

6. Place culture plate containing domes into a 37 °C incubator for 30 minutes. Inverting the culture plate during incubation will promote proper dome formation and prevent the settling of organoids toward the culture dish surface.
7. Add appropriate organoid growth culture medium for continued culture.

### Thin Coating for Pluripotent Stem Cell Expansion

Cultrex UltiMatrix RGF BME is qualified as a coating substrate for pluripotent stem cell expansion. The following protocol describes a basic protocol for use.

1. Thaw Cultrex UltiMatrix RGF BME as stated above.
2. Mix Cultrex UltiMatrix RGF BME by slowly pipetting solution up and down; be careful not to introduce air bubbles.
3. Dilute Cultrex UltiMatrix RGF BME to desired concentration in cold serum-free medium. Empirical determination of the optimal coating dilution for your application may be required. A final protein concentration of 80-90  $\mu$ g/mL is recommended for the propagation of stem cells.
4. Add a sufficient amount of solution to cover the entire area onto growth surface. A volume of 300  $\mu$ L per  $\text{cm}^2$  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.

**Note:** Coated plates can be prepared in advance. Follow the procedures below:

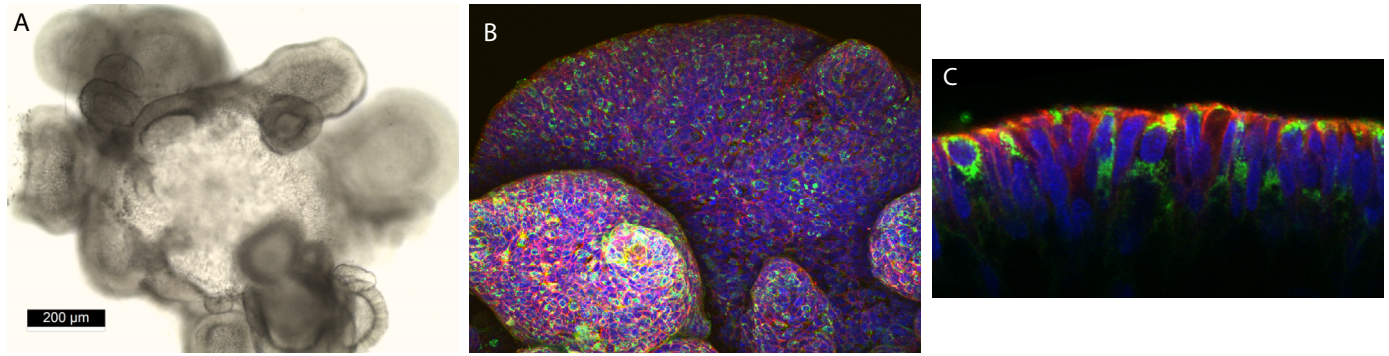
- a. Follow Steps 1-4; then seal the plates with Parafilm® and store for up to two weeks in a refrigerator at 2-8 °C.
- b. Prior to use, incubate coated plates at room temperature for one hour.
- c. Continue with Step 6.

### Thick Gel Method

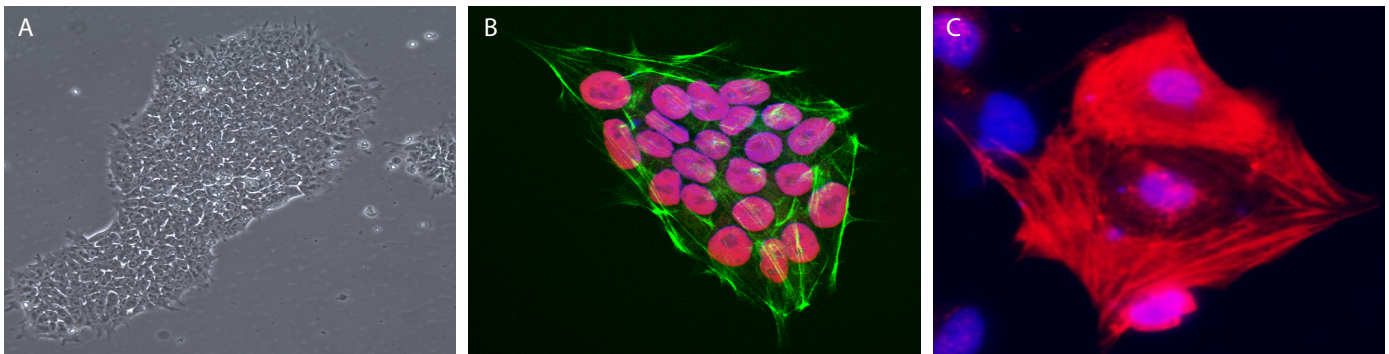
A thick gel is needed for applications such as endothelial cell formation of capillary-like structures (Tube Formation Assay), the differentiation of rat aorta tissue into capillary-like structures (Aortic Ring Assay), epithelial organoid formation, or tumor organoid formation. The following protocol describes a basic protocol for creating a thick coat of Cultrex UltiMatrix RGF BME.

1. Thaw Cultrex UltiMatrix RGF BME as stated above.
2. Mix Cultrex UltiMatrix RGF BME by slowly pipetting solution up and down; be careful not to introduce air bubbles.
3. Pipette 200-300  $\mu$ L per  $\text{cm}^2$  onto the growth surface.
4. Place coated object at 37 °C for 30 minutes.
5. Coated objects are ready for use.

## DATA EXAMPLES



**Figure 2. Human Descending Colon Organoids Grown in Cultrex UltiMatrix RGF BME.** Adult stem cells isolated from human descending colon were embedded in Cultrex UltiMatrix RGF BME and cultured in growth medium for 30 days prior to imaging. **A)** Brightfield image of descending colon organoid showing tissue invagination and epithelial layer formation. **B, C)** Descending colon organoids were stained with Chromogranin A (green; R&D Systems®, Catalog # MAB90981), to visualize intestinal enteroendocrine cells, and counterstained for E-Cadherin (red; R&D Systems, Catalog # AF748) and DAPI (blue; Tocris™, Catalog # 5748).



**Figure 3. Cultrex UltiMatrix RGF BME Supports Culture and Differentiation of Human Induced Pluripotent Stem Cells (hiPSC).** iBJ6 hiPSCs were cultured on plates coated with Cultrex UltiMatrix RGF BME diluted 1:100 (~ 100 µg/mL) using DMEM. **A)** Representative brightfield image of iBJ6 (Passage 7) human induced pluripotent stem cell colony cultured. **B)** Immunofluorescent staining of hiPSC colonies for Oct-3/4 (red; R&D Systems®, Catalog # AF1759), F-actin (green), and DAPI (blue; Tocris, Catalog # 5748). **C)** iBJ6 iPSCs were differentiated using the StemXVivo™ Cardiomyocyte Differentiation Kit (R&D Systems, Catalog # SC032B) and stained for expression for Troponin T (red; R&D Systems, Catalog # MAB1874) and DAPI (blue).