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White Paper

Basement Membrane Basics

Expert Methods for Handling, Coating, and Embedding

Principles & Methods for Using Basement Membrane Extracts in Cell Culture

Substrates that mimic the extracellular matrix (ECM) have been shown to be imperative for robust cell growth, optimal cell health, and as a method to direct the behavior and structural formation of cells in vitro. Basement membrane extracts (BME) are a commonly used ECM substrate for 2-D and 3-D cell culture, including pluripotent stem cell expansion and differentiation as well as spheroid and organoid formation.

While BME is being utilized across many applications, it is well known that the quality and behavior of cells cultured in BME can be greatly impacted by the handling and coating methods. This paper provides guidance for working with BME hydrogels, such as Cultrex™ BME, and highlights the preferred coating or embedding methods specific to culturing stem cells, spheroids, and organoids. In addition to selecting a coating method and handling your matrix properly, choosing a quality matrix makes a difference for your cells (Figure 1).

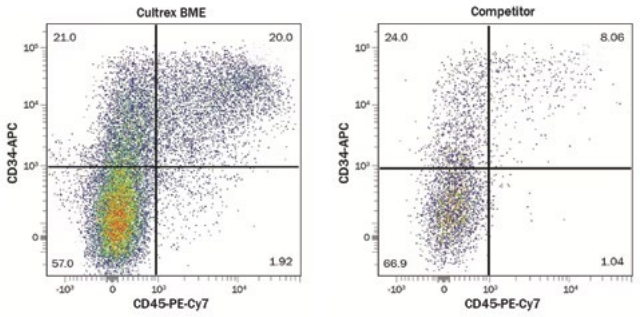
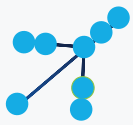


FIGURE 1. Cultrex BME Improves Hematopoietic Stem Cell Differentiation from Induced Pluripotent Stem Cells (iPSCs). Human iPSCs were grown for a minimum of 2 passages on either Cultrex Stem Cell Qualified RGF BME (Catalog # 3434-005-02) or a leading competitor's matrix, prior to creating embryoid bodies for hematopoietic stem cell differentiation. Cells pre-cultured on Cultrex Stem Cell Qualified RGF BME, showed a greater efficiency to develop into hematopoietic stem cells (CD34+, CD45+). Data courtesy of the Verneris Laboratory at the University of Colorado.

ECM Proteins in BME



Laminin

Heterotrimeric, noncollagenous glycoprotein that interacts with integrins, dystroglycan, and other receptors.

Fibronectin

Large modular glycoprotein that is found as a polymeric fibrillar network in the extracellular matrix.

Collagen IV

Composed of two alpha1(I) chains and one alpha2(I) chain that spontaneously forms a triple helix scaffold.

Vitronectin

A large glycoprotein that forms a single chain monomer and is important for cell adhesion.

Entactin

A secreted, monomeric glycoprotein that serves as a major linking component of basement membranes.

Proteoglycans

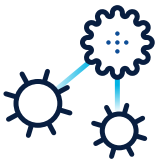
Type 1 transmembrane proteins capable of carrying heparan sulfate (HS) and chondroitin sulfate glycosaminoglycans and binding proteins in the extracellular matrix.

What are Basement Membrane Extracts?

Basement Membrane Extracts (BME), also referred to as basement membrane matrices or basement membrane hydrogels, are commercially available hydrogels consisting of ECM proteins, including collagen IV, laminin, entactin, and heparan sulfate proteoglycan. The specific mixture of ECM proteins plays an important role in directing cell behavior and health, providing not only a critical growth substrate but also by an optimized environment for cell-to-cell interactions that facilitate *in vivo*-like 2-D and 3-D growth dynamics (Figure 2). Cell behavior *in vitro* is greatly impacted by the concentration and integrity of the hydrogel. Understanding the intricacies of working with basement membranes, including proper handling and coating methods, can greatly improve the robustness, consistency, and behavior of the cells in culture.

BME is purified from Engelbreth-Holm-Swarm (EHS) mouse sarcoma tissue and, when maintained at 2–8 °C, takes the form of a liquid hydrogel. At ≥ 18 °C, BME rapidly solidifies into a matrix that provides a scaffold for cell adherence and cell organization. In addition, BME and similar hydrogels can provide soluble microenvironmental cues, such as growth factors and hormones, that can help define the growth and organization of the 3-D tissue.

Additional purification steps can be applied to generate Reduced Growth Factor (RGF) versions of BME, which are desired for applications where more defined culture conditions are needed, such as pluripotent stem cell expansion and differentiation or organoid cell culture.



CELL-MATRIX INTERACTION

3-D cultures grown in BME recreate *in vivo*-like interactions between cell structures and the extracellular matrix, which impact cell function and behavior. BME is still frequently used for 2-D cultures, such as the expansion of pluripotent stem cells.

CELL MORPHOLOGY

Cells grown in 3-D using BME are dimensionally unrestricted for growth, emulating development and migration *in vivo*. Cells grown in 2-D using BME are constrained to the x-y plane, limiting growth as well as cell-cell and cell-matrix interactions.

CELL-CELL INTERACTION

As with other extracellular matrices, BME hydrogels enable cells to engage in crosstalk with neighboring cells. This is a critical component for 3-D cell culture but also for cells grown in 2-D.

GRADIENTS AND PENETRATION

Growing cells in 3-D using BME enables the gradients and tissue penetration of intrinsic and extrinsic growth factors and compounds. This is beneficial for establishing drug efficacy, infiltration, and bystander killing effects within a complex 3-D culture microenvironment.

CELL POLARITY

Despite restricted apical-basal polarity in 2-D, BME can influence robust growth and cell homogeneity. For 3-D cultures, BME enables the formation of tissue with more *in vivo*-like cell polarity.

FIGURE 2. 2-D and 3-D growth dynamics

Choosing The Best Coating Method For Your Application

When working with BMEs, it is important to understand the various coating methods that can be employed and the impact they can have on your cell culture. The method of use is dictated by multiple factors, including the type of starting material (single cells or tissue), the adherence requirements of your cell or tissue type of interest, and the need for a 2-D or 3-D culture system. In the following sections, more details and descriptions of the coating methods are described.

As an example of coating method selection, see how distinct methods for using Cultrex BME are employed depending on whether the goal is expansion or differentiation of embryonic or induced pluripotent stem cells (PSCs). A thin coat of Cultrex BME provides a base substrate that supports feeder-free expansion of PSCs. For differentiation of stem cells into terminal cell types, a sandwich coating method, where cells are embedded between two layers of BME is sometimes employed. This method is ideal for cultures that require additional adherence support (i.e., contractile iPSC-derived cardiomyocytes). Additional methods, such as the thick coat, embedded, and dome embedded methods are typically used for more complex 3-D culture systems.

Basic Handling Tips for Cultrex™ BME

Basement membrane hydrogels have unique physical properties. They are in a liquid-state at 2–8 °C and polymerize when brought to room temperature. While a liquid, Cultrex BME is easily manipulated and diluted. However, even a temporary exposure above 2-8 °C can result in subtle physical state changes, ultimately impacting the health and behavior of the cell culture system. Best practice for working with Cultrex BME, or similar EHS-derived hydrogels, is to keep it on ice at all times. Below are some additional tips for handling Cultrex BME.

- **Keep all stock solutions at -80 °C for long term storage (≥ 1 month).** Thaw frozen Cultrex BME on ice in a 2–8 °C refrigerator overnight. This allows for ample thawing while maintaining the product at a stable, cold temperature.
- **Aliquot Cultrex BME into working amounts.** Store aliquots at -80 °C for long term storage and avoid storing Cultrex BME at 2–8 °C for > 1 day.
- **Work quickly and always on ice.** Use pre-chilled supplies and tools, such as pipette tips. Always dilute Cultrex BME with ice-cold media or buffer. Cultrex BME will gel if the temperature is ≥ 18 °C.

TABLE 1. BME Coating Methods, Applications, & Recommended Matrices

Method	Description	Application	Recommended Matrix
Thin Coat	Cells are grown on top of a thin layer of Cultrex BME	• Primary Cell Propagation • iPSC Expansion • Cell Invasion Assays	Cultrex UltiMatrix RGF BME (Catalog # BME001-05) Cultrex Stem Cell Qualified RGF BME (Catalog # 3434-010-02) Cultrex RGF BME (Catalog # 3433-005-01) Cultrex BME (Catalog # 3432-005-01)
Thick Coat	Cells are grown on top of a thick layer of Cultrex BME	• Spheroid Culture • Organoid Culture • Endothelial Vessels • Tube Formation • Aortic Rings	Cultrex UltiMatrix RGF BME (Catalog # BME001-05) Cultrex RGF BME (Catalog # 3433-005-01) Cultrex 3-D RGF BME (Catalog # 3445-005-01)
Sandwich	Cells are cultured between two thin layers of Cultrex BME	• iPSC Differentiation • MSC Expansion	Cultrex UltiMatrix RGF BME (Catalog # BME001-05) Cultrex Stem Cell Qualified RGF BME (Catalog # 3434-005-02) Cultrex 3-D RGF BME (Catalog # 3445-005-01)
Embedded - Layer	Cells are cultured while embedded in Cultrex BME	• Spheroid Culture • Organoid Culture • Invasion and Migration Assays	Cultrex UltiMatrix RGF BME (Catalog # BME001-05) Cultrex 3-D RGF BME (Catalog # 3445-005-01) Cultrex RGF BME, Type 2 (Catalog # 3533-005-02) Cultrex RGF BME, Type R1 (Catalog # 3433-005-R1)
Embedded - Dome	Cells are cultured while embedded in Cultrex BME and plated into cell culture vessel as domed structures	• Organoid culture	Cultrex UltiMatrix RGF BME (Catalog # BME001-05) Cultrex RGF BME, Type 2 (Catalog # 3533-005-02) Cultrex RGF BME, Type R1 (Catalog # 3433-005-R1)

*RGF signifies reduced growth factor formulation

Thin Coat Method

The thin coating method for Cultrex BME provides an adherent ECM substrate for cell proliferation and maintenance. It is commonly used for the expansion of embryonic and induced pluripotent stem cells but can be broadly applied to cell lines or primary cells. Benefits of this coating method are its simplicity to set-up and its accessibility for immunocytochemistry and cell imaging downstream.

Briefly, this method entails diluting ice-cold Cultrex BME in ice-cold cell culture media, pipetting the solution into a culture dish, and solidifying the matrix by incubating at 37 °C for at least 30 minutes. Cells suspended in culture media are seeded into the well where they adhere to the thin coat Cultrex BME.

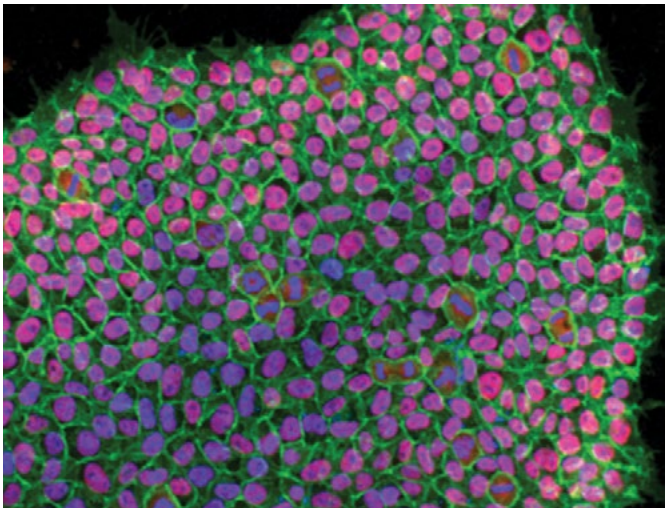
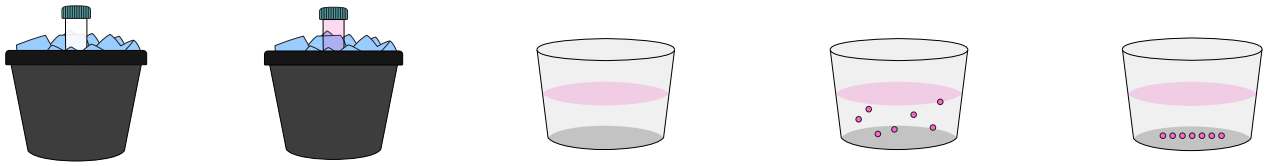


FIGURE 3. Induced Pluripotent Stem Cells Cultured Using the Thin Coat Method. Image shows a colony of iPSCs cultured using Cultrex Stem Cell Qualified RGF BME (Catalog # 3433-005-01). Cells were stained for SOX1 (red), E-Cadherin (green), and DAPI (blue).

METHOD 1. Thin Coat Method



1. Thaw Cultrex BME on ice in a 2 - 8 °C refrigerator overnight.
2. Dilute ice-cold Cultrex BME with ice- cold culture media.

Note: *Recommended starting dilution of 1:100, but this may vary depending on application and final protein concentration must be empirically determined by the researcher based on cell type.*

3. Pipette Cultrex BME into the wells/plates. Incubate plates for 30-60 minutes to enable matrix solidification at 37 °C.

Note: *Once solidified it is important to make sure the Cultrex BME coating does not dry out. Move to next step immediately or add more buffer, seal plate with parafilm, and store at 2-8 °C until ready for culture.*

4. Aspirate coating. Seed cells suspended in culture media onto thin coat Cultrex BME plates.
5. Place plate in 37 °C incubator for cell attachment.

Thick Coating Method

Compared to the thin coating method, the thick coating method uses a higher concentration of undiluted Cultrex BME which is preferential for the formation of 3-D structures, such as aortic rings and endothelial cell tube formation for angiogenesis research.

This method involves plating ice-cold Cultrex BME directly into the cell culture vessel. The matrix is solidified by incubating the plate at 37 °C for 30-60 minutes. Cells suspended in culture media are seeded into the well where they adhere to the thick coat Cultrex BME.

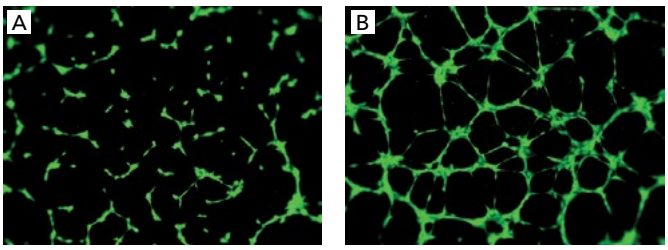
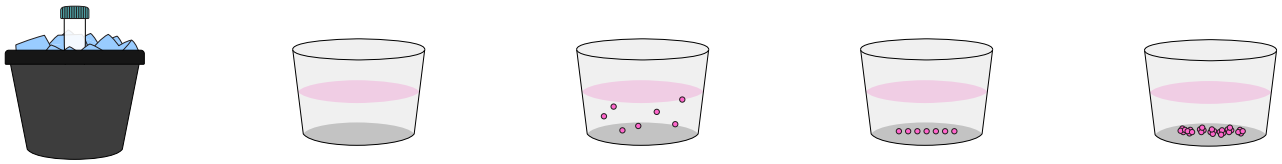


FIGURE 4. Thick Coating Method is Used for Endothelial Cell Tube Formation. Human Umbilical Vein Endothelial Cells (HUVECs) were harvested, counted and diluted in either Endothelial Cell Basal Medium (which does not contain serum or angiogenic factors), or Endothelial Cell Growth Medium, containing all supplements and growth factors necessary to support HUVEC expansion. HUVECs were seeded (1 x 10^4 cells/well) onto gelled Cultrex RGF BME (Catalog # 3433-005-01) and thereafter cultured for four hours at 37 °C and 5% CO2. Representative images of Calcein AM (Catalog # 4892-010-01) stained cells grown in Basal Medium (A) and Growth Medium (B).

METHOD 2. Thick Coat Method



1. Thaw Cultrex BME on ice in a 2–8 °C refrigerator overnight.
2. Pipette Cultrex BME into the wells/plates. Incubate plates for 30-60 minutes to enable matrix solidification at 37 °C.
3. Seed cells suspended in culture media onto thick coat Cultrex BME plates. Allow cells to settle.
4. Culture the cells.
5. Evaluate cell expansion or differentiation.

Note: *Once solidified it is important to make sure the Cultrex BME coating does not dry out. Move to next step immediately or seal plate with parafilm until ready for culture.*

Sandwich Coating Method

The sandwich coating method for Cultrex BME provides a more complex ECM microenvironment for cell growth and differentiation. It is also beneficial for cultures where a larger adherence area is needed to accommodate mechanical stress.

To begin the sandwich coating method, coat the plate using the thin coat method. After the cells have adhered to the plate, overlay the cells with another layer of diluted Cultrex BME. Cells are allowed to culture until the desired phenotype is observed. While this process takes slightly more work than the thin coating method, the cells are fully embedded into the matrix providing a more rigid 3-D scaffolding environment.

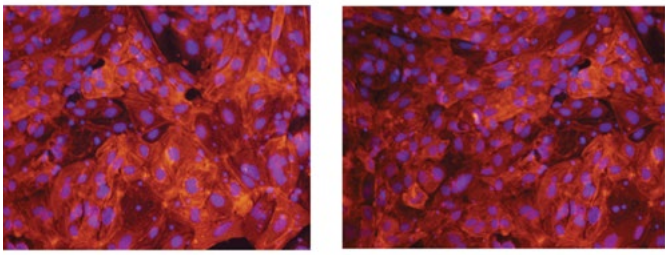
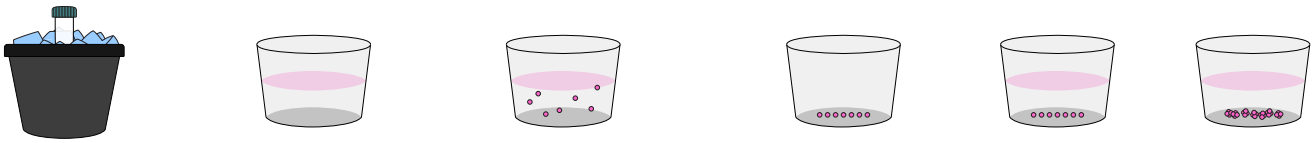


FIGURE 5. Differentiation of Pluripotent Stem Cells into Cardiomyocytes using the Sandwich Coating Method. Human induced pluripotent stem cells were differentiated into cardiomyocytes utilizing the sandwich method. Aside from visually observable contracting cells, commitment to the cardiomyocyte cell fate was evaluated by Human Cardiac Troponin T (red). Nuclei were counterstained with DAPI (blue; Catalog # 5748).

METHOD 3. Sandwich Coating Method



1. Thaw Cultrex BME on ice in a 2–8 °C refrigerator overnight. Dilute ice-cold Cultrex BME with ice-cold culture media.
2. Pipette diluted Cultrex BME into the wells/plates. Incubate plates for 60 minutes to enable matrix solidification at 37 °C.
3. Seed cells suspended in culture media onto thin coat Cultrex BME plates.

   Allow cells to settle and attach.
4. Add second layer of diluted ice-cold Cultrex BME. Place plates into a 37 °C incubator for 30 minutes to enable matrix solidification.
5. Culture the cells.
6. Evaluate cell expansion or differentiation.

Note: *Dilutions may vary by cell type and assay. For example, for iPSC differentiation into cardiomyocytes, the bottom layer dilution is 1:40, top layer dilution is 1:60.*

Note: *Once solidified it is important to make sure the Cultrex BME coating does not dry out. Move to next step immediately or seal plate with parafilm until ready for culture.*

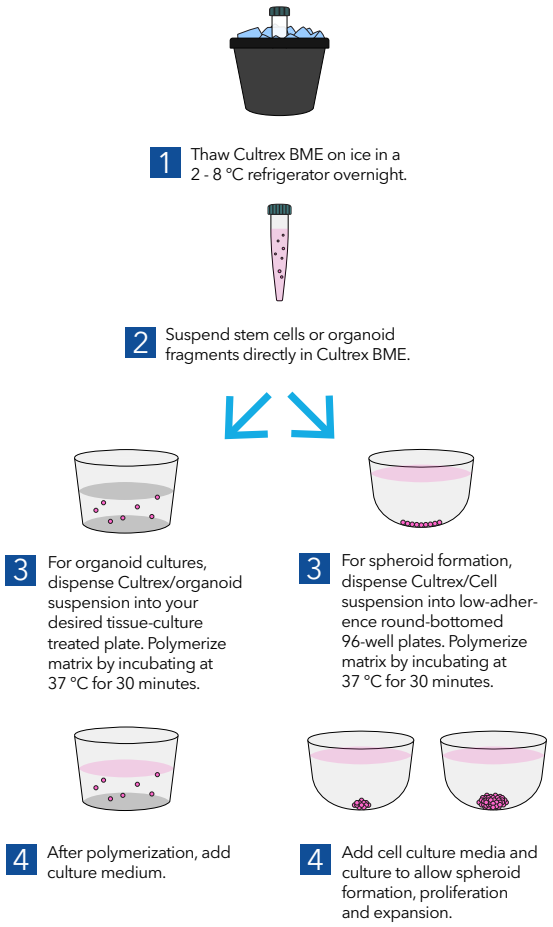
Embedded: Layer Method and Dome Method

The embedded methods result in the full surrounding of cells in the Cultrex BME hydrogel, which promotes cell expansion and maturation in a more *in vivo*-like microenvironment compared to the other coating methods. The most common applications for using the embedding method are: 1) for spheroid cell invasion or migration assays for modelling oncogenesis and cancer cell metastasis, and 2) the formation of organoids, or mini-organs such as intestine, brain, liver, kidney, and pancreas.

Layer Method Quick Protocol

Briefly, ice-cold Cultrex BME is mixed directly with cancer cell lines, primary tissue fragments, or induced pluripotent stem cells. This mixture is plated directly into the desired cell culture vessel. The matrix is solidified by incubating the plate at 37 °C for 30 minutes. Media is then added on top of the solidified Cultrex BME/cell mixture. This method is commonly used for organoid formation, spheroid formation, and for cell invasion and migration assays.

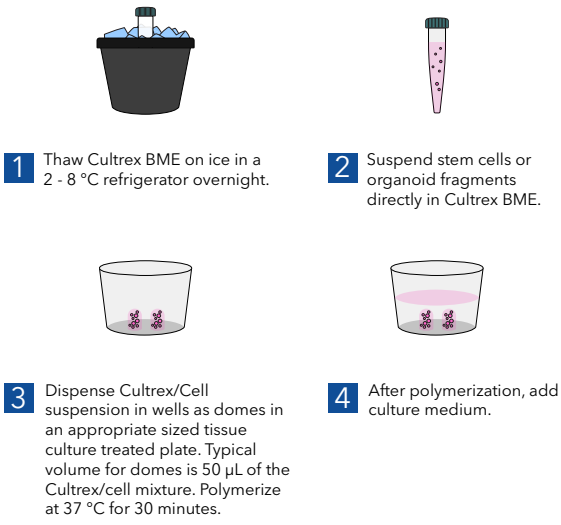
METHOD 4. Layer Method Quick Protocol



Dome Method Quick Protocol

Briefly, ice-cold Cultrex BME is mixed directly with organoids (1 x 10^4 organoids/mL or 500 organoids/50 µL). Dispense 50 µL of the Cultrex BME/organoid mixture in the center of each well of a 24-well plate or arrange domes placing 6 to 8 domes in a well of a 6-well plate. Incubate the plate at 37 °C for 30 minutes to solidify the matrix, then add appropriate cell culture media to each well.

METHOD 5. Dome Method Quick Protocol



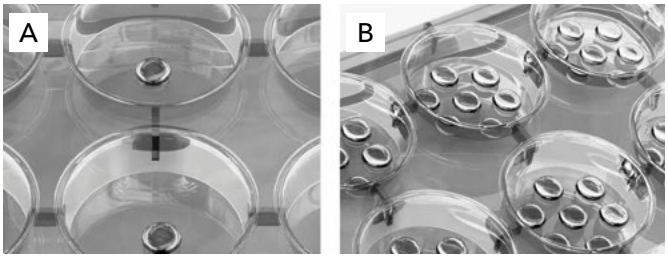


FIGURE 6. Placement of Cultrex UltiMatrix RGF BME/Organoid Mixture in a 24-well or 6-well Plate. (A) Placement of Cultrex UltiMatrix RGF BME/organoid mixture in the center of the well of a 24-well plate or (B) placement of multiple domes within a well of a 6-well plate.

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