TECHNICAL GUIDE

# Basement Membrane Basics
## Principles and Guidance for Using Cultrex® BME in 2D and 3D Cell Culture

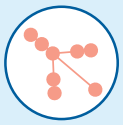
Substrates that mimic the extracellular matrix (ECM) in culture have been shown to be imperative for robust cell growth, optimal health, and as a method to direct the behavior and structural formation of cells *in vitro*. Basement membrane extract (BME) is a commonly used ECM substrate for 2D and 3D cell culture, including pluripotent stem cell expansion and differentiation as well as spheroid and organoid formation. While BME is being utilized across a wide breadth of applications, it is well known that the quality and behavior of cells cultured in BME can be greatly impacted by handling and coating methods.

This paper provides guidance for working with BME hydrogels, such as Cultrex BME, and highlights **basic handing principles** and **preferred coating or embedding methods** for culturing stem cells, spheroids, and organoids.

### After reading this you will:

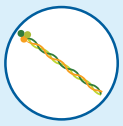
- ✓ Know the basic elements and applications of BME and ECM proteins
- ✓ Understand handling principles for successful use of Cultrex BME
- ✓ Be able to determine the correct coating method for your desired cell culture
- ✓ Immediately implement specific coating protocols

### Basement Membrane Proteins Utilized as 2D and 3D Cell Culture Scaffolds



**Laminin**
Is a heterotrimeric, noncollagenous glycoprotein that interacts with integrins, dystroglycan, and other receptors.

**Fibronectin**
Is a large modular glycoprotein that is found as a polymeric fibrillar network in the extracellular matrix.



**Collagen IV**
Is a 185 kDa molecule composed of two alpha1(I) chains and one alpha2(I) chain that spontaneously forms a triple helix scaffold.

**Vitronectin**
Is a large glycoprotein that forms a single chain monomer and is important for cell adhesion.

**Entactin**
Is a 150 kDa, secreted, monomeric glycoprotein that serves as a major linking component of basement membranes.

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

bio-techne®

# What is Basement Membrane Extract?

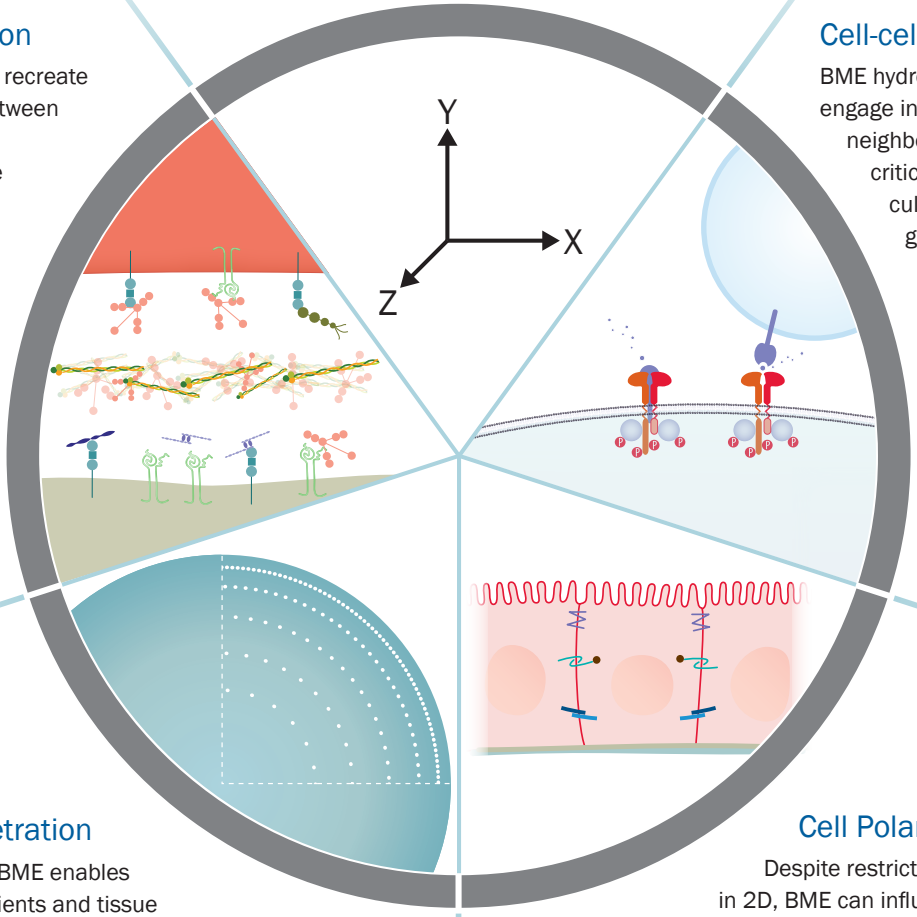
Basement Membrane Extract (BME), also referred to as basement membrane matrix or basement membrane hydrogel, is a commercially available hydrogel consisting of ECM proteins, including collagen, laminin, entactin, and heparan sulfate proteoglycan. The mixture of ECM proteins within the BME plays an important role in directing cell behavior and health, providing not only a critical growth substrate but also by an optimized environment for cell-cell interactions that facilitate *in vivo*-like 2D and 3D growth dynamics (Figure 1).

## Cell Morphology

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

## Cell-matrix Interaction

3D cultures grown in BME recreate *in vivo*-like interactions between cell structures and the extracellular matrix. These interactions impacts cell function and behavior. This complexity is limited to the x-y plane in 2D cultures, however BME is still critical for sensitive 2D cultures, such as expansion of pluripotent stem cells.




## Cell-cell Interaction

BME hydrogel enable cells to engage in crosstalk with neighboring cells. This is a critical component for 3D cell culture but also for cells grown in 2D.

## Gradients and Penetration

Growing cells in 3D using BME enables the establishment of 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 3D culture microenvironment.

## Cell Polarity

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

**Figure 1.** Key features of extracellular matrices in cell 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. However, at ≥18 °C BME solidifies rapidly into a matrix that provides a substrate for cell adherence and cell organization. In addition to being a scaffold, 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 3D 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.

As a manufacturer of Cultrex BME, a widely used basement membrane hydrogel, and other purified ECM proteins, we understand that cell behavior *in vitro* is greatly impacted by the concentration and integrity of the hydrogel. Understanding the intricacies of working with basement membranes, including the proper handling and coating methods, can greatly improve the robustness, consistency, and behavior of the cells in 2D and 3D culture.

### Cultrex® BME Details

- ✓ Soluble form of basement membrane purified from Engelbreth-Holm-Swarm (EHS) tumor
- ✓ Polymerizes at ≥18 °C to form a reconstituted basement membrane
- ✓ Is composed of laminin, collagen IV, entactin, and heparan sulfate proteoglycans
- ✓ Provides a physiological alternative for culturing cells in an *in vitro* environment
- ✓ Tested to USP <71> sterility guidelines

## Basics for Handling Cultrex BME—Keep it Cold!

Basement membrane hydrogels, such as Cultrex BME, have unique physical properties, they are in a liquid-state at 2–8 °C and solidify when brought to room temperature or 37 °C. While a liquid, Cultrex BME can be easily manipulated and diluted, however their sensitivity to changes in physical state due to temperature changes does need to be kept in mind when working with them. Fluctuations or temporary exposure to temperatures above 2–8 °C can potentially result in subtle changes in Cultrex BME's physical state. These changes could ultimately impact 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 cold or on ice at all times. Below are some basic working tips for handling Cultrex BME

### When working with 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 -80 °C for long term storage
- ✓ Avoid storing Cultrex BME at 2–8 °C for > 1 day
- ✓ Cultrex BME will gel if temperature is ≥ 18 °C, so work quickly and always on ice and with pre-chilled supplies and tools, such as pipette tips.
- ✓ Always dilute Cultrex BME with ice-cold cell culture media

# Choosing the Best Coating Methods for Your Application

Cultrex BME is a versatile soluble form of basement membrane that can be applied to a variety of cell culture conditions. However, 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 2D or 3D culture system.

As an example, distinct methods for using Cultrex BME are employed for the 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 used for more complex 3D culture systems (Table 1).

In the following sections, more detailed methods and descriptions of Cultrex BME coating methods are described.

| Method | Description | Application | Recommended Cultrex BME |
|---|---|---|---|
| **Thin Coat** | Cells are grown on top of a thin layer of Cultrex BME | • Primary cell propagation<br>• iPSC expansion<br>• Cell invasion assays | Stem Cell Qualified Cultrex RGF BME (Catalog # 3434-005-01)<br>Cultrex RGF BME (Catalog # 3433-005-01)<br>Cultrex BME (Catalog # 3432-005-01) |
| **Thick Coat** | Cells are on top of a thick layer of Cultrex BME | • Tube formation<br>• Aortic rings<br>• Endothelial Vessels<br>• Spheroid and Organoids | Cultrex RGF BME (Catalog # 3433-005-01)<br>Cultrex 3-D RGF BME (Catalog # 3445-005-01) |
| **Sandwich** | Cells are cultured in-between two thick layers of Cultrex BME | • iPSC differentiation<br>• MSC expansion | Stem Cell Qualified Cultrex RGF BME (Catalog # 3434-005-01)<br>Cultrex RGF BME (Catalog # 3433-005-01) |
| **Embedded - Layer** | Cells are cultured while embedded in Cultrex BME | • Spheroid culture<br>• Invasion and Migration Assays<br>• Organoid culture | Cultrex 3D RGF BME (Catalog # 3445-005-01)<br>Cultrex RGF BME, Type 2 (Catalog # 3533-005-02)<br>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 RGF BME, Type 2 (Catalog # 3533-005-02)<br>Cultrex RGF BME, Type R1 (Catalog # 3433-005-R1) |

**Table 1.** Methods and descriptions of coating methods using Cultrex BME

# 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.

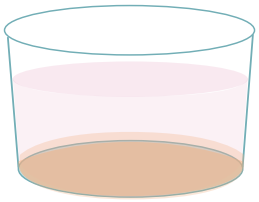
Briefly, this method entails diluting ice-cold Cultrex BME in ice-cold cell culture media, pipetting the solution into a culture dish, and solidifying the matrix by incubating at 37 °C for at least 30 minutes. Cells suspended in culture media are seeded into the well where they adhere to the thin coat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

## Thin Coat Quick Protocol

1. Thaw Cultrex BME on ice in a 2–8 °C refrigerator overnight.

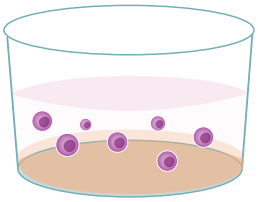
2. Dilute ice-cold Cultrex BME with ice-cold culture media.

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

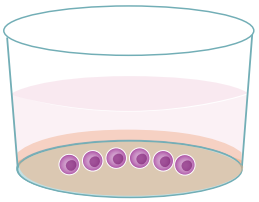


3. Pipette Cultrex BME into the wells/plates. Incubate plates for 30–60 minutes to enable matrix solidification at 37 °C.

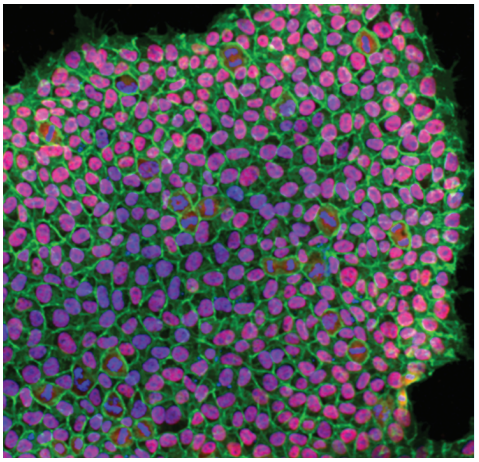
   ***Note:*** *Once solidified it is important to make sure the Cultrex BME coating does not dry out. Move to next step immediately or seal plate with parafilm until ready for culture.*



4. Seed cells suspended in culture media onto thin coat Cultrex BME plates.



5. Place plate in 37 °C incubator for cell attachment.



**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).

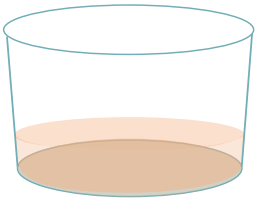
# Thick Coating Method

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

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

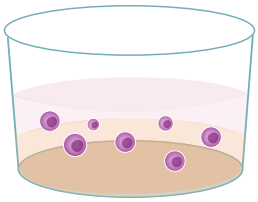
## Thick Coating Quick Protocol

1. Thaw Cultrex BME on ice in a 2–8 °C refrigerator overnight.

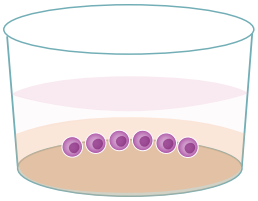


2. Pipette Cultrex BME into the wells/plates. Incubate plates for 60 minutes to enable matrix solidification.

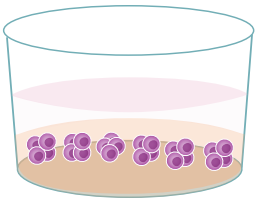
   ***Note**: Once solidified it is important to make sure the Cultrex BME coating does not dry out. Move to next step immediately or seal plate with parafilm until ready for culture.*



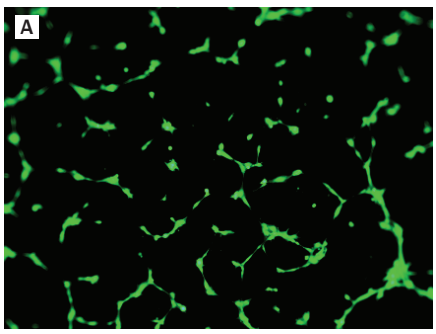
3. Seed cells suspended in culture media onto thin coat Cultrex BME plates. Allow cells to settle.

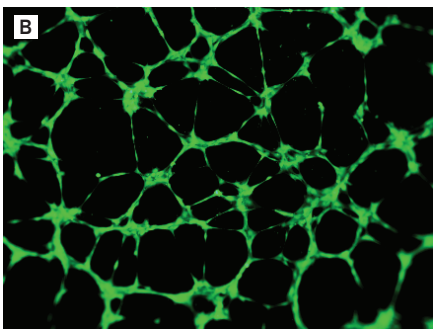


4. Culture the cells.



5. Evaluate cell expansion or differentiation.




**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 % $CO_2$. Representative images of Calcein AM (Catalog # 4892-010-01) stained cells grown in Basal Medium (**A**) and Growth Medium (**B**).

# Sandwich Coating Method

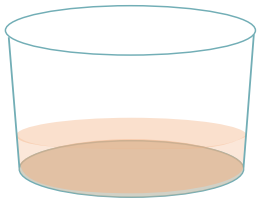
The sandwich coating method for Cultrex BME provides a more complex ECM microenvironment for cell growth and differentiation in a more *in vivo*-like 3D environment. It is also beneficial for cultures where a larger adherence area is needed to accommodate mechanical stress (i.e., contractile iPSC-derived cardiomyocytes).

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. Following solidification of that layer, media is added to the well and the 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 3D scaffolding environment.

## Sandwich Coat Quick Protocol

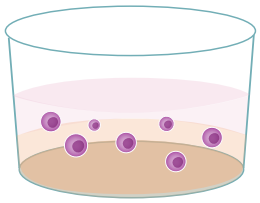
1. Thaw Cultrex BME on ice in a 2-8 °C refrigerator overnight.

   Dilute ice-cold Cultrex BME with ice-cold culture media.
   ***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.*

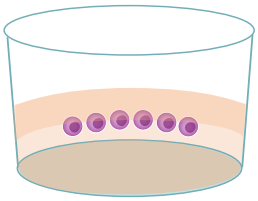


2. Pipette diluted Cultrex BME into the wells/ plates. Incubate plates for 60 minutes to enable matrix solidification.

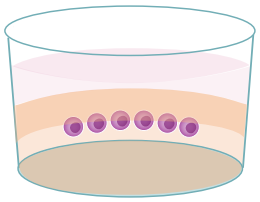
   ***Note**: Once solidified it is important to make sure the Cultrex BME coating does not dry out. Move to next step immediately or seal plate with parafilm until ready for culture.*



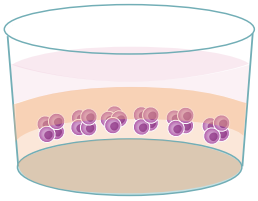
3. Seed cells suspended in culture media onto thin coat Cultrex BME plates.
   Allow cells to settle and attach.



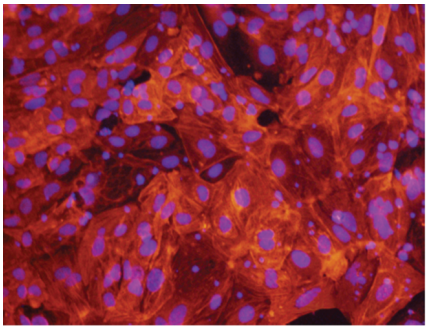
4. Aspirate medium and add second layer of diluted ice-cold Cultrex BME. Place plates into a 37 °C incubator for 30 minutes to enable matrix solidification.

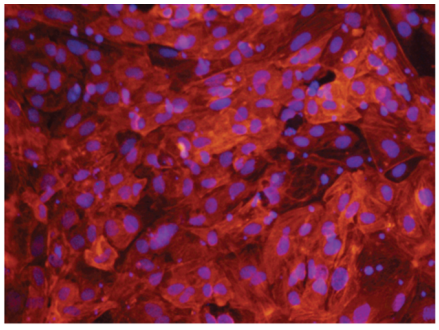


5. Add medium and culture the cells.



6. Evaluate cell expansion or differentiation.





**Differentiation of Pluripotent Stem Cells into Cardiomyocytes using the Sandwich Coating Method.** JOY6 (left panel) and iBJ6 (right panel) human induced pluripotent stem cells were differentiated into cardiomyocytes using the StemXVivo® Cardiomyocyte Differentiation Kit (Catalog # SC032B), which utilizes the sandwich method. Aside from visually observable contracting cells, commitment to the cardiomyocyte cell fate was evaluated by for Human Cardiac Troponin T (red). Nuclei were counterstained with DAPI (blue; Catalog # 5748).

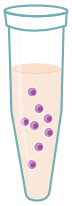
# Embedded Methods

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.

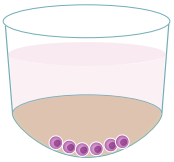
## Layer Methods Quick Protocol

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.

1 Thaw Cultrex BME on ice in a 2–8 °C refrigerator overnight.

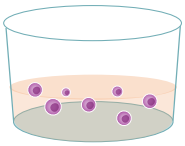


2 Suspend stem cells or organoid-fragments directly in Cultrex BME.

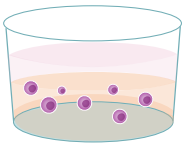


3 For spheroid formation, dispense Cultrex/Cell suspension into low-adherence round-bottomed 96-well plates. Polymerize matrix by incubating at 37 °C for 30 minutes.

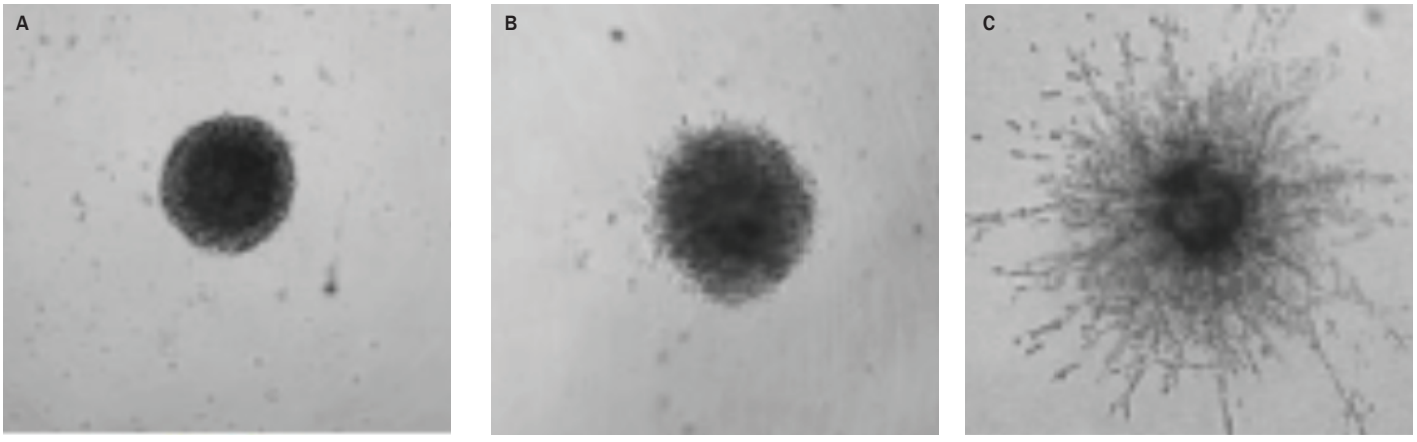
4 Add cell culture media and culture to allow spheroid formation, proliferation and expansion.



3 For organoid cultures, dispense in Cultrex/organoid suspension into your desired tissue-culture treated plate. Polymerize matrix by incubating at 37 °C for 30 minutes.



4 After polymerization, add culture medium.

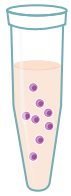



**Embedding Spheroids in Cultrex BME for Cell Invasion and Migration Assays.** MDA-MB-231 (3,000 cells/well) formed spheroids in low adhesion plates for 72 hours. Then spheroids were embedded in **A**) No matrix, **B**) 10 mg/mL Cultrex BME, or **C**) 10 mg/mL Cultrex BME and 1 mg/mL Collagen I. After hydrogel polymerization, DMEM, 10% FBS was added to each well to promote a chemotactic response over a 96 hour period. Note that Cultrex BME and ECM choice affects the extent of cell invasion and migration.

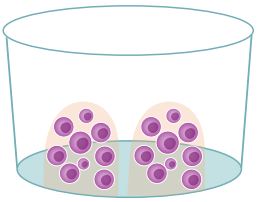
## Dome Method Quick Protocol

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 (Figure ) 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.

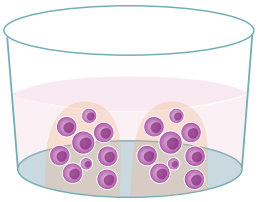
1. Thaw Cultrex BME on ice in a 2–8 °C refrigerator overnight.



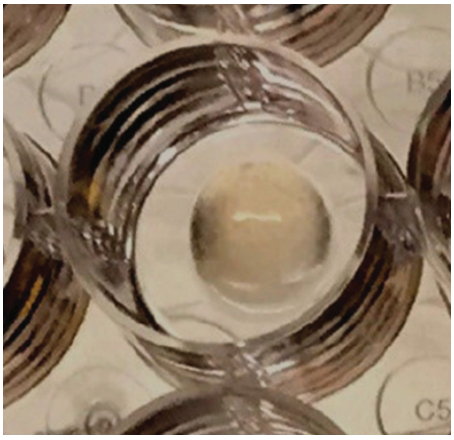
2. Suspend cells or organoid-fragments directly in Cultrex BME.



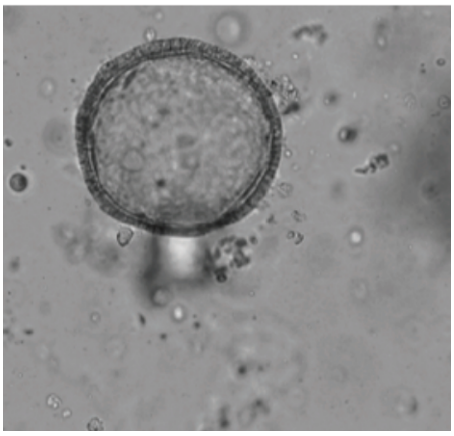
3. Dispense Cultrex/Cell suspension in wells as domes in an appropriate sized tissue-culture treated plate. Typical volume for domes is 50 uL of the Cultrex/cell mixture. Polymerize at 37 °C for 30 minutes.

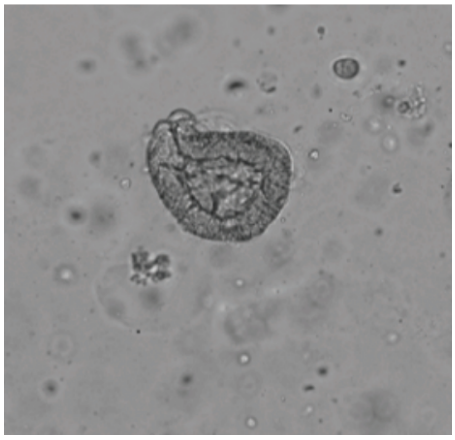


4. After polymerization, add culture medium.



**Organoids Plated Using the Dome Method.** Representative dome placement for a Cultrex RGF BME, Type 2/organoid mixture in the center of the well of a 24-well plate. Human gastric organoids were utilized in this experiment.



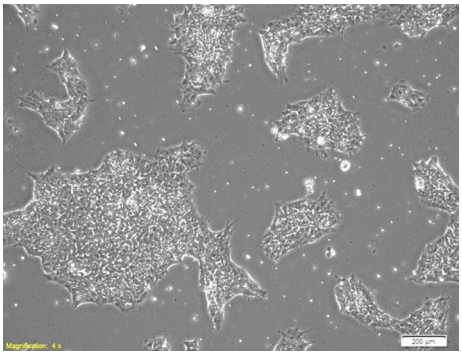


**Undifferentiated Human Gastric Organoids Embedded in Domes of Cultrex BME.** Representative brightfield images of undifferentiated human gastric organoids that were cultured using Cultrex RGF BME (Catalog # 3533-005-02) following the Human Gastric Organoid Culture Protocol.

# Cultrex Use In Real Life: Application Data and Publications

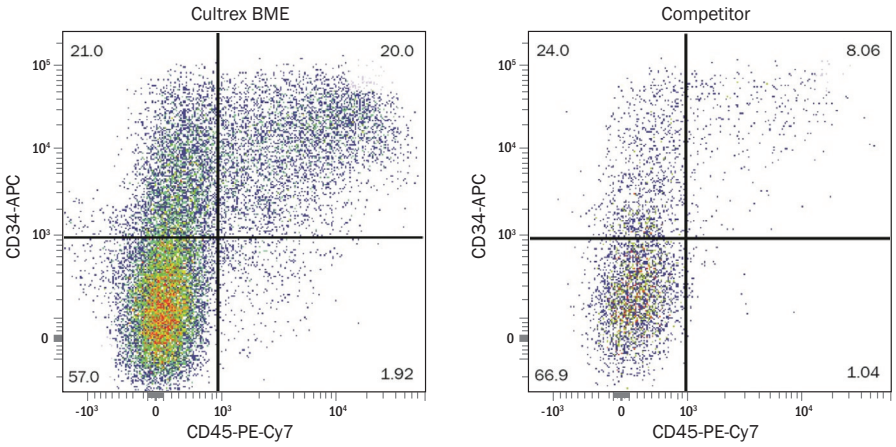
## Application Data from the Field

### Harvard Stem Cell Core



**Colonies of iPSCs Grown on Cultrex Stem Cell Qualified RGF BME.** Erythroblasts, reprogrammed into induced pluripotent stem cells (iPSCs), were cultured on plates coated with Cultrex Stem Cell Qualified RGF BME (Catalog # 3434-005-02). Example colonies are shown at 4X magnification. *Data courtesy of the Harvard Stem Cell Core.*

### University of Colorado




**Cultrex Stem Cell Qualified RGF BME Improves Hematopoietic Stem Cell Differentiation from Induced Pluripotent Stem Cells (iPSCs).** iPSCs were grown for a minimum of 2 passages on either Cultrex Stem Cell Qualified RGF BME (R&D Systems, 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.*

## Peer Reviewed Publications using Organoid Qualified Cultrex BME, Type 2

**Hubrecht Institute**

Artegiani, B. *et al.* (2019) Probing the Tumor Suppressor Function of BAP1 in CRISPR-Engineered Human Liver Organoids. Cell Stem Cell **24**:927.

**Stanford University**

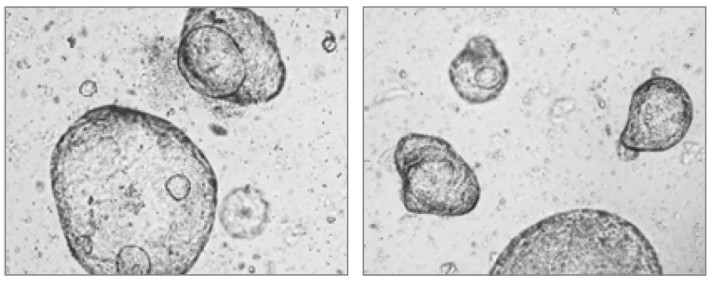
Co, J. *et al.* (2019) Controlling Epithelial Polarity: A Human Enteroid Model for Host-Pathogen Interactions. Cell Reports **26**:2509.

**Gurdon Institute**

Broutier, L. *et al.* (2017) Human Primary Liver Cancer-derived Organoid Cultures for Disease Modelling and Drug Screening. Nature **23**:1424.

**University of California, Los Angeles**

Phan, N. *et al.* (2019) A Simple High-throughput Approach Identifies Actionable Drug Sensitivities in Patient-derived Tumor Organoids. Communications Biology **2**:78.



**Human Pancreatic Organoids Cultured in Cultrex Reduced Growth Factor BME, Type 2.** Human pancreatic progenitor cells were cultured in Cultrex RGF BME, Type 2 (Catalog # 3533-001-02) and were differentiated into pancreatic organoids.

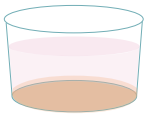
# Protocol Quick Guides

## Thin Coat Quick Protocol

❶ Thaw Cultrex BME on ice in a 2–8 °C refrigerator overnight.

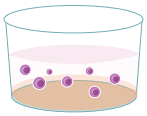
❷ Dilute ice-cold Cultrex BME with ice-cold culture media.

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

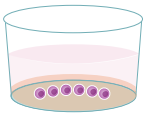


❸ Pipette Cultrex BME into the wells/plates. Incubate plates for 30–60 minutes to enable matrix solidification at 37 °C.

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



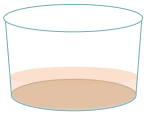
❹ Seed cells suspended in culture media onto thin coat Cultrex BME plates.



❺ Place plate in 37 °C incubator for cell attachment.

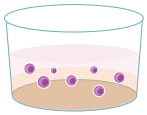
## Thick Coating Quick Protocol

❶ Thaw Cultrex BME on ice in a 2–8 °C refrigerator overnight.

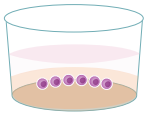


❷ Pipette Cultrex BME into the wells/plates. Incubate plates for 60 minutes to enable matrix solidification.

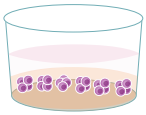
***Note:*** *Once solidified it is important to make sure the Cultrex BME coating does not dry out. Move to next step immediately or seal plate with parafilm until ready for culture.*



❸ Seed cells suspended in culture media onto thin coat Cultrex BME plates.
Allow cells to settle.



❹ Culture the cells.

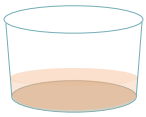


❺ Evaluate cell expansion or differentiation.

## Sandwich Coat Quick Protocol

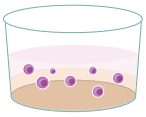
❶ Thaw Cultrex BME on ice in a 2-8 °C refrigerator overnight.

Dilute ice-cold Cultrex BME with ice-cold culture media.
***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.*

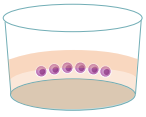


❷ Pipette diluted Cultrex BME into the wells/plates. Incubate plates for 60 minutes to enable matrix solidification.

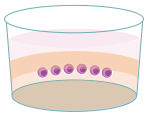
***Note:*** *Once solidified it is important to make sure the Cultrex BME coating does not dry out. Move to next step immediately or seal plate with parafilm until ready for culture.*



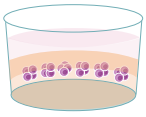
❸ Seed cells suspended in culture media onto thin coat Cultrex BME plates.
Allow cells to settle and attach.



❹ Aspirate medium and add second layer of diluted ice-cold Cultrex BME. Place plates into a 37 °C incubator for 30 minutes to enable matrix solidification.



❺ Add medium and culture the cells.



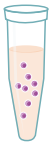
❻ Evaluate cell expansion or differentiation.

*More Quick Protocols >>>*

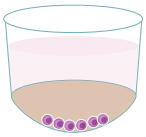
# Protocol Quick Guides *continued*

## Layer Methods Quick Protocol

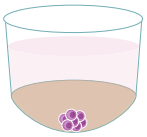
❶ Thaw Cultrex BME on ice in a 2–8 °C refrigerator overnight.

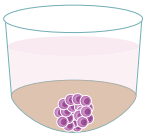


❷ Suspend stem cells or organoid-fragments directly in Cultrex BME.

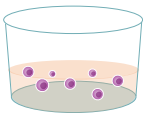


❸ For spheroid formation, dispense Cultrex/Cell suspension into low-adherence round-bottomed 96-well plates. Polymerize matrix by incubating at 37 °C for 30 minutes.

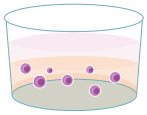




❹ Add cell culture media and culture to allow spheroid formation, proliferation and expansion.



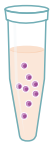
❸ For organoid cultures, dispense in Cultrex/organoid suspension into your desired tissue-culture treated plate. Polymerize matrix by incubating at 37 °C for 30 minutes.



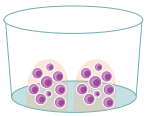
❹ After polymerization, add culture medium.

## Dome Method Quick Protocol

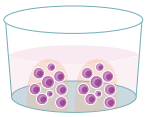
❶ Thaw Cultrex BME on ice in a 2–8 °C refrigerator overnight.



❷ Suspend cells or organoid-fragments directly in Cultrex BME.



❸ Dispense Cultrex/Cell suspension in wells as domes in an appropriate sized tissue-culture treated plate. Typical volume for domes is 50 uL of the Cultrex/cell mixture. Polymerize at 37 °C for 30 minutes.



❹ After polymerization, add culture medium.

View Cultrex BME Portfolio | rndsystems.com/cultrex

R&D SYSTEMS NOVUS BIOLOGICALS TOCRIS proteinsimple ACD™

bio-techne®

**Global** bio-techne.com info@bio-techne.com TEL +1 612 379 2956 **North America** TEL 800 343 7475
**Europe | Middle East | Africa** TEL +44 (0)1235 529449 **China** info.cn@bio-techne.com TEL +86 (21) 52380373

For research use or manufacturing purposes only. Trademarks and registered trademarks are the property of their respective owners.