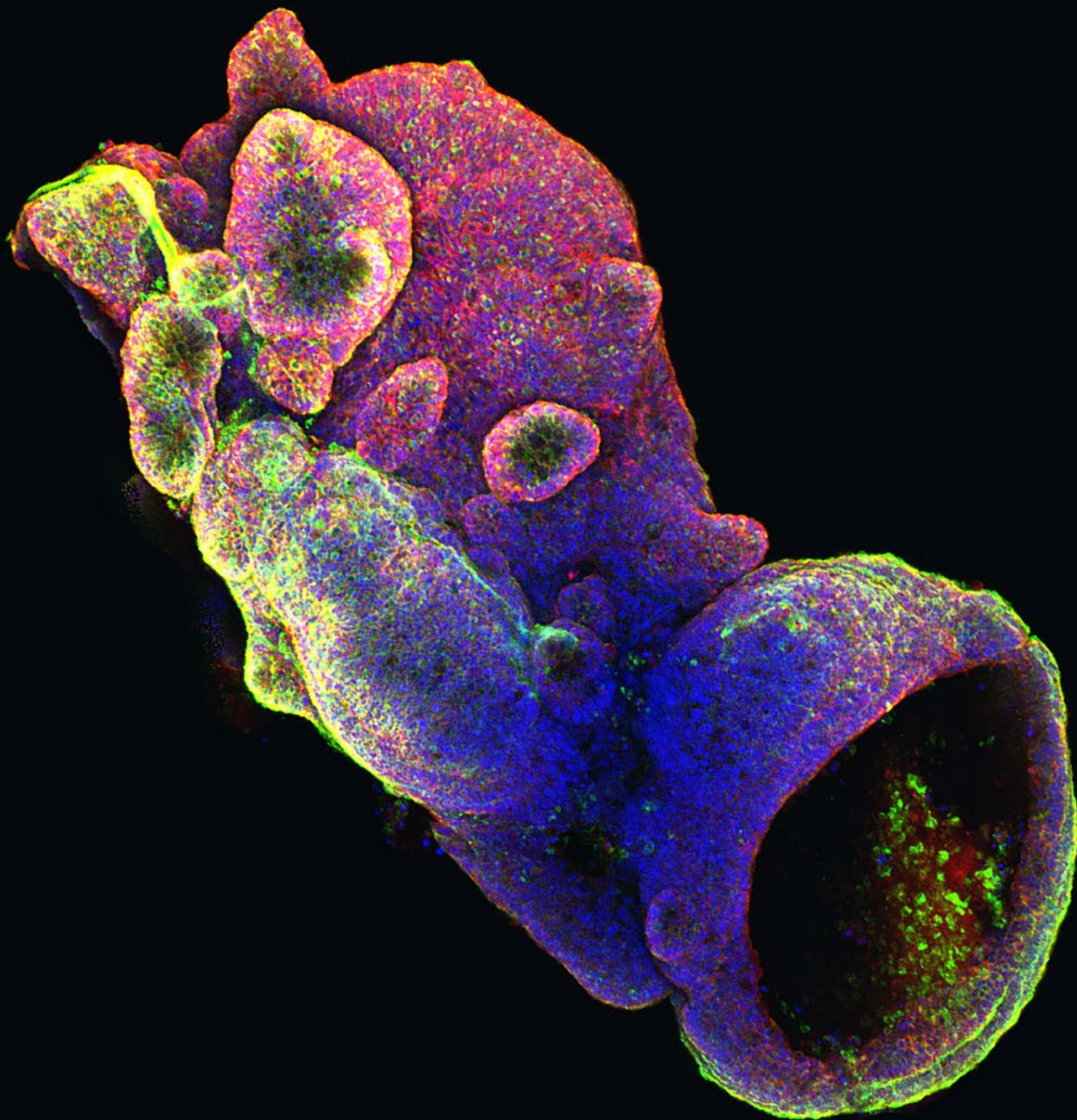


CULTREX™ PRODUCTS FOR PHYSIOLOGIC CELL CULTURE



biotechne®

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INTRODUCTION TO PHYSIOLOGIC CELL CULTURE

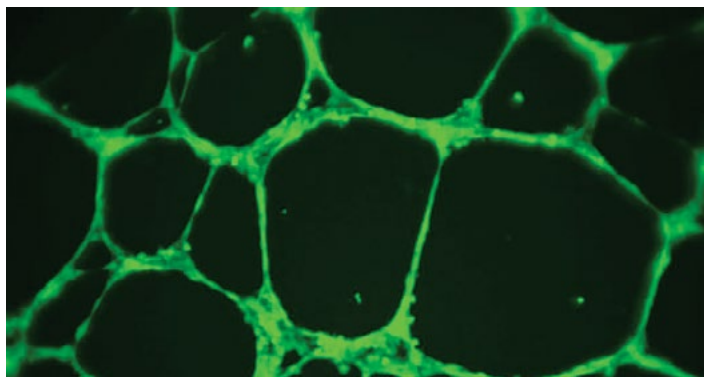
R&D Systems products for physiologic cell culture recreate tissue microenvironments to elicit *in vivo* structure and function for cells *in vitro*. These tools for 2-D and 3-D cell culture are designed, developed, produced, qualified, and supported by our Cultrex™ laboratory. The product portfolio includes several unique types of Basement Membrane Extract (BME) matrices containing different compositions of extracellular matrix (ECM) proteins. These matrices are qualified for specific applications, such as 3-D, stem cell, spheroid, and organoid cultures. Other purified ECM proteins offered include Laminin I, Collagen I, Collagen IV, Vitronectin, and Fibronectin, as well as the poly-amino acids, Poly-D-Lysine, Poly-L-Lysine and Poly-L-Ornithine. Our organoid culture products provide organoid-qualified basement membrane matrices for intestinal, liver, colon, pancreatic, and breast organoids, among others.

CULTREX™ BASEMENT MEMBRANE EXTRACT FOR 2-D & 3-D TISSUE CULTURE

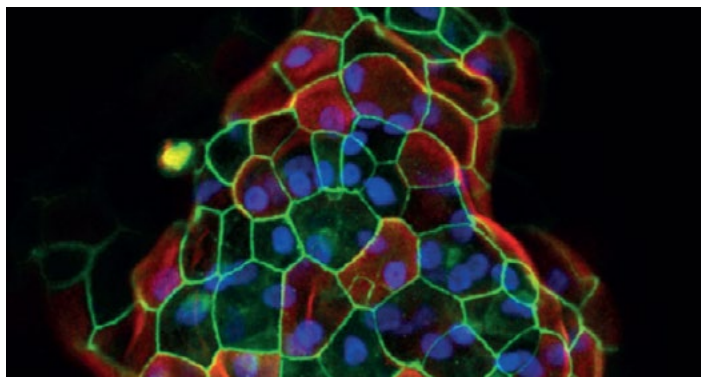
Cultrex Basement Membrane Extract (BME) is a soluble form of basement membrane purified from Engelbreth-Holm-Swarm (EHS) tumors. This extract provides a natural extracellular matrix hydrogel that polymerizes at 37 °C to form a reconstituted basement membrane. Basement membranes are continuous sheets of specialized extracellular matrix that form an interface between endothelial, epithelial, muscle, or neuronal cells and their adjacent stroma. They play an essential role in tissue organization by influencing cell adhesion, migration, proliferation, and differentiation. The major components of BME include laminin, collagen IV, entactin, and heparan sulfate proteoglycans.

Cultrex BME for 2-D and 3-D culture is offered in multiple formats to support application-specific needs. Cultrex Reduced Growth Factor (RGF) BME, which is processed to reduce matrix-associated growth factors, is ideal for research that requires a more defined model system.

NAME	SIZE	CATALOG #
Cultrex Basement Membrane Extract	1 mL	3432-001-01
	5 mL	3432-005-01
	2 x 5 mL	3432-010-01
Cultrex Reduced Growth Factor Basement Membrane Extract	1 mL	3433-001-01
	5 mL	3433-005-01
	2 x 5 mL	3433-010-01
Cultrex 3-D Culture Matrix™, Basement Membrane Extract, Reduced Growth Factor	1 mL	3445-001-01
	5 mL	3445-005-01
	2 x 5 mL	3445-010-01



Tube Formation of HUVECs Using Cultrex BME Reduced Growth Factor. Human Umbilical Vein Endothelial Cells (HUVEC) were cultured using **Cultrex BME Reduced Growth Factor** (R&D Systems, Catalog # 3433-005-01) for four hours at 37 °C and 5% CO₂. Tube formation was visualized by labelling HUVECs with 2 μM **Calcein AM** (R&D Systems, Catalog # 4892-010-K).



Differentiation of Organoids into Hepatocytes using Cultrex RGF BME, Type 2. Human liver organoids were differentiated in **Cultrex RGF BME, Type 2** (R&D Systems, Catalog # 3533-005-02) for 11 days and then evaluated for expression of hepatocyte markers using immunocytochemistry. Liver organoid cells show expression of Albumin (red) and were counterstained for Zona Occludens-1 (green) and Hoechst (blue).

Learn more | rndsystems.com/cultrex

CULTREX™ EXTRACELLULAR MATRIX PROTEINS

Cultrex Extracellular Matrix (ECM) Proteins provide a defined substrate for cell adhesion in culture. Many proteins of the ECM interact with cells via cell surface integrin family receptors. The resulting focal contacts are important for the maintenance of tissue architecture and for supporting a variety of cellular processes. ECM molecule/integrin binding may initiate a complex network of signal transduction cascades that, depending on the context, play an important role in cell spreading, migration, proliferation, and differentiation during embryogenesis, wound healing, and tumor development. To accommodate a variety of different models, we have developed a full range of ECM component proteins including Laminin I, Collagen I, Collagen IV, Fibronectin, and Vitronectin. These include purified proteins that have been qualified for 3-D or stem cell cultures.

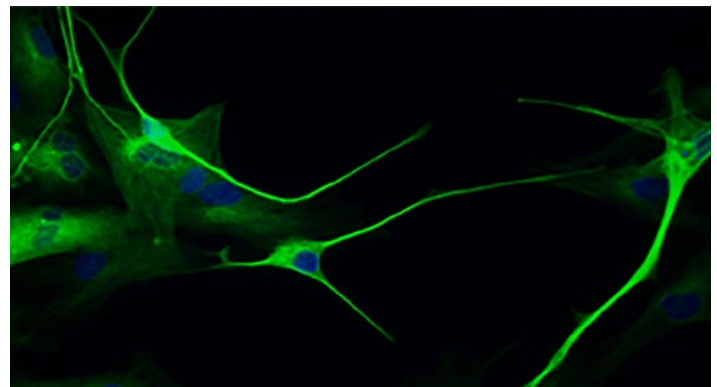
NAME	SIZE	CATALOG #
Cultrex Mouse Laminin I, PathClear®	1 mg	3400-010-02
Cultrex Mouse Collagen IV	1 mg	3410-010-02
Cultrex Human Fibronectin, PathClear®	1 mg	3420-001-01
Cultrex Human Vitronectin, PathClear®	50 µg	3421-001-01
Cultrex Rat Collagen I (5 mg/ml)	1 ml	3440-005-01
Cultrex Rat Collagen I (5 mg/ml)	20 ml	3440-100-01

NAME	SIZE	CATALOG #
Cultrex Bovine Collagen I (5 mg/ml)	1 ml	3442-005-01
Cultrex Bovine Collagen I (5 mg/ml)	10 ml	3442-050-01
Cultrex Rat Collagen I, Lower Viscosity (3 mg/ml)	35 ml	3443-100-01
Cultrex 3-D Culture Extract Laminin I	30 mg	3446-005-01
Cultrex 3-D Culture Extract Rat Collagen I	20 ml	3447-020-01

CULTREX™ POLY-AMINO ACIDS

Normal tissue culture-treated (TCT) plastic exhibits a net negative charge which is the result of physical and/or chemical modifications. Due to variations in plasma membrane composition, this surface is not optimal for cell adhesion. Poly-amino acids are highly charged, synthetic polymers made up of many repeating units of an amino acid that may be applied to normal TCT plastic or glass surfaces to provide a positively charged coating for enhanced cell adhesion. Cultrex Poly-L-Lysine and Poly-D-Lysine contain polymers in the 70,000-150,000 Da range and promote the growth and differentiation of a variety of neuronal cell lines. Poly-L-Ornithine contains polymers in the 30,000 - 70,000 Da range, and is frequently used either alone, or in combination with Fibronectin or Laminin-I, to enhance the attachment and differentiation of various types of neuronal and neural stem cells. All of these products are provided ready-to-use at a concentration of 0.01%.

NAME	SIZE	CATALOG #
Cultrex Poly-L-Ornithine	100 ml	3436-100-01
Cultrex Poly-D-Lysine	100 ml	3439-100-01
Cultrex Poly-L-Lysine	100 ml	3438-100-01



Human Neural Stem Cells Differentiated into Neurons on Cultrex ECM. Human neural stem cells (NSCs) were cultured with [Cultrex Poly-L-Ornithine](#) (R&D Systems, Catalog # 3436-100-01) and [Cultrex Stem Cell Qualified Laminin I](#) (R&D Systems, Catalog # 3400-010-03). Human NSCs were differentiated into neurons and characterized by expression of beta-III-tubulin using an Anti-beta-III Tubulin antibody (green). Cell nuclei were detected with Hoechst 33342 (blue). Magnification: 20X. Image provided by Dr. Meritxell Huch.

CULTREX™ ORGANOID AND STEM CELL CULTURE PRODUCTS

We have developed a full line of products that are designed and qualified for expanding, passaging, and differentiating cultures to support reproducible and robust organoid and stem cell models. Products include organoid and stem cell-qualified matrices (alternatives to Matrigel® and Geltrex®), serum-free media supplements, a wide selection of bioactive recombinant proteins, and small molecules. These products, along with essential reagents like Cultrex Organoid Harvesting Solution, will allow your lab to take your stem cell and 3-D culture research to the next level.

NEW! CULTREX™ ULTIMATRIX REDUCED GROWTH FACTOR BASEMENT MEMBRANE EXTRACT

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, elevated protein concentration, and robust clarity and purity. These compositional and concentration enhancements translate into substantial performance benefits that make Cultrex UltiMatrix RGF BME an ideal cell scaffolding matrix with proven efficacy in organoid cell culture, induced pluripotent stem cell expansion and differentiation, spheroid formation, and other 2-D and 3-D culture applications.

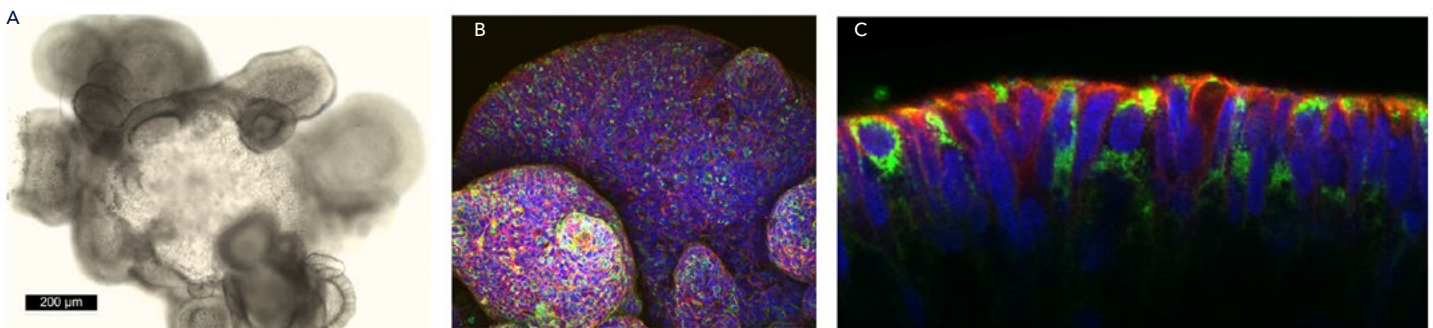
KEY BENEFITS

- Tensile strength optimized for 3D applications
- Qualified for organoid and pluripotent stem cell culture
- Verified for dome formation
- Consistent lot-to-lot performance

PRODUCT FEATURES

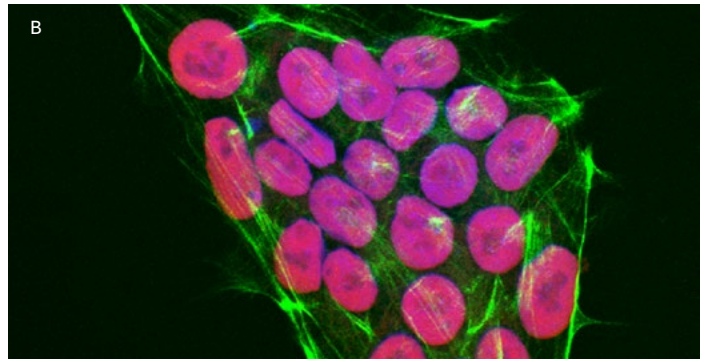
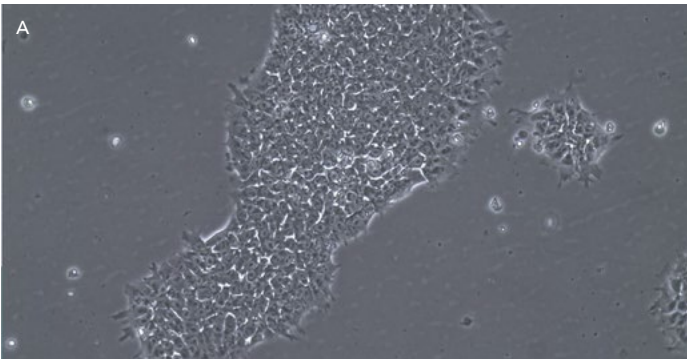
- High protein concentration (10-12 mg/mL)
- Enriched in entactin (> 8% of total protein)
- Reduced Growth Factor (RGF) formulation
- Low endotoxin profile (< 7 EU/mL)

NAME	SIZE	CATALOG #
Cultrex UltiMatrix Reduced Growth Factor Basement Membrane Extract	1 ML	BME001-01
	5 ML	BME001-05
	2 X 5 ML	BME001-10

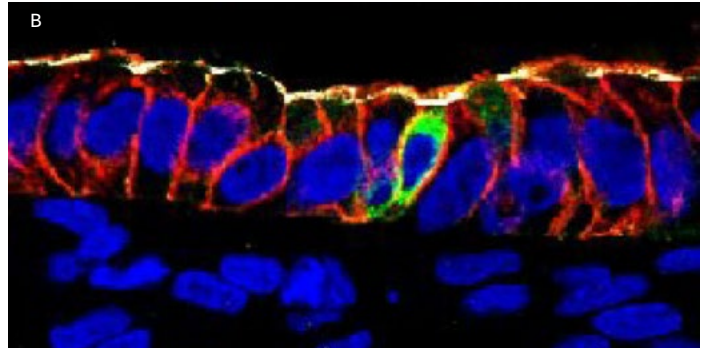
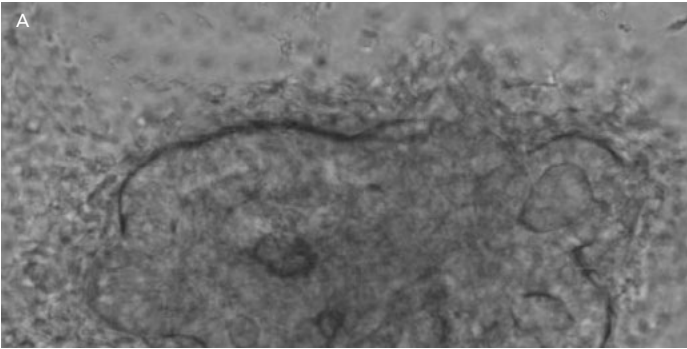


Human Descending Colon Organoids Grown in Cultrex UltiMatrix RGF BME. Adult stem cells isolated from human descending colon were embedded in [Cultrex UltiMatrix RGF BME](#) (R&D Systems, Catalog # BME001) and cultured in growth medium for 30 days prior to imaging. **A**) Brightfield image of descending 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; R&D Systems, Catalog # 5748).

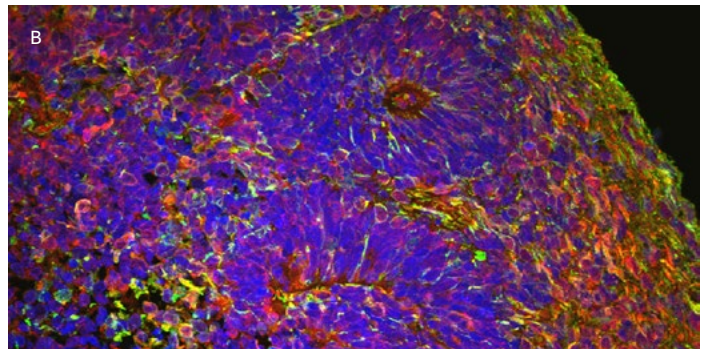
Learn more | rndsystems.com/ultimatrix



Cultrex UltiMatrix BME Supports the Culture of Human Induced Pluripotent Stem Cells (hiPSC). iBJ6 hiPSCs were cultured on plates coated with **Cultrex UltiMatrix RGF BME** (R&D Systems, Catalog # BME001) diluted 1:100 (~100 µg/mL) using DMEM. **A)** Representative brightfield image of iBJ6 (Passage 7) human induced pluripotent stem cell colony culture. **B)** Immunofluorescent staining of hiPSC colonies using F-actin show the typical F-actin stress fibers (green) that define hiPSC colony edges as well as nuclear expression of the pluripotency marker OCT3 (red).



iPSC-derived Intestinal Organoids Grown in Cultrex UltiMatrix BME. Human iPSCs were embedded in **Cultrex UltiMatrix RGF BME** (R&D Systems, Catalog # BME001) and cultured in growth medium. hiPSC-derived intestinal organoids cultured for 13 days were imaged using **(A)** brightfield microscopy or processed and **(B)** stained for Chromogranin A (green), Villin (white), E-Cadherin (red), and DAPI (blue).



iPSC-derived Cerebral Organoids Grown in Cultrex UltiMatrix BME. **A)** Representative image of iPSC-derived cerebral organoids (day 30) cultured using **Cultrex UltiMatrix RGF BME** (R&D Systems, Catalog # BME001). **B)** Cerebral organoid stained for beta III-tubulin (green), Prox1 (red) and DAPI (blue).

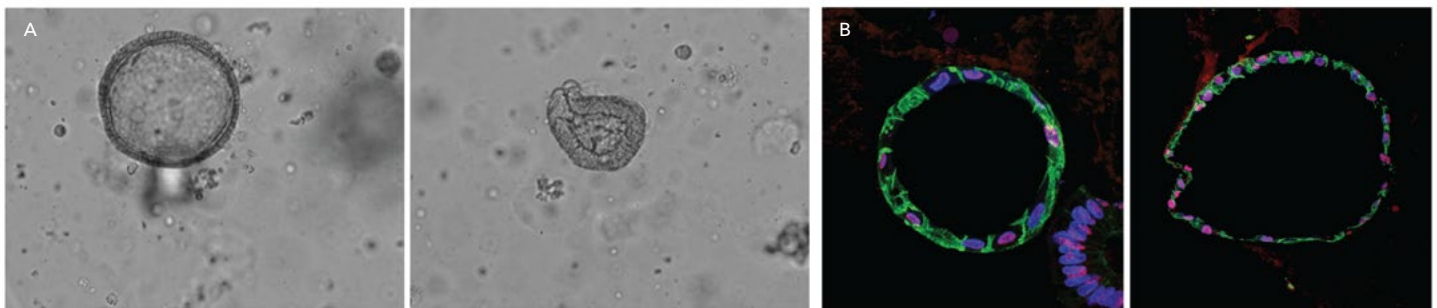
Learn more | rndsystems.com/ultimatrix

CULTREX™ ORGANOID-QUALIFIED BASEMENT MEMBRANE EXTRACT

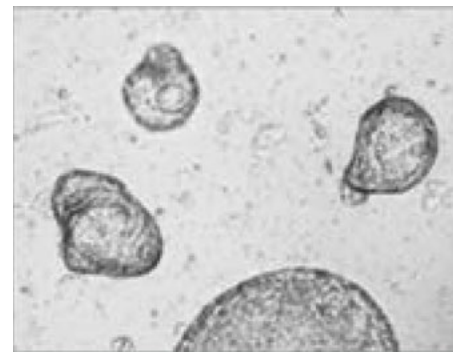
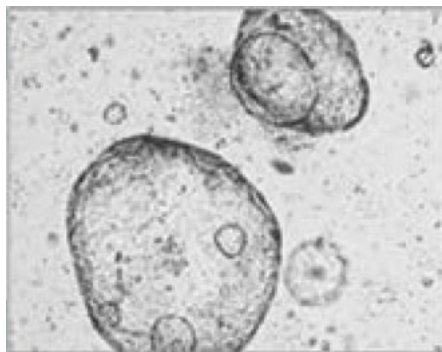
Our exclusive organoid-qualified matrices were developed and designed for robust and reproducible expansion, passaging, and differentiation of organoids. Products include Cultrex Basement Membrane Extract, Type 2 and Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2. These qualified matrices undergo extensive evaluation in-house for take rate, proliferation, and morphology of enteric organoids embedded.

NAME	SIZE	CATALOG #
Cultrex Basement Membrane Extract, Type 2	1 mL	3532-001-02
	5 mL	3532-005-02
	2 x 5 mL	3532-010-02

NAME	SIZE	CATALOG #
Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2	1 mL	3533-001-02
	5 mL	3533-005-02
	2 x 5 mL	3533-010-02



Brightfield Images and Immunohistochemistry of Undifferentiated Human Gastric Organoids. (A) Representative brightfield images of human gastric organoids that were cultured using **Cultrex RGF BME, Type 2** (R&D Systems, Catalog # 3533-005-02) as a scaffolding matrix. (B) Human gastric organoids were cultured using **Cultrex RGF BME, Type 2** (R&D Systems, Catalog # 3533-005-02) as a scaffolding matrix. Undifferentiated colon organoids were stained using the **Human/Mouse E-Cadherin Antibody** (green; R&D Systems, Catalog # AF748), the **Human HOXB7 Antibody** (red; R&D Systems, Catalog # MAB8040), and counterstained with **DAPI** (blue; R&D Systems, Catalog # 5748).



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

Learn more | rndsystems.com/organoids

ORGANOID AND 3-D CELL CULTURE REAGENTS

Complement the use of Cultrex BME and ECM proteins for culturing organoids with media supplements, growth factors, small molecules, and cell-specific marker antibodies from R&D Systems and Tocris Biosciences.

BASE MEDIA COMPONENTS		
PRODUCT	DESCRIPTION	CATALOG #
N-2 MAX Supplement	Similar to N-2	AR009
N21-MAX Supplement	Similar to B27	AR008
N21-MAX Vitamin A Free Supplement	Similar to B27 - Vitamin Free	AR012
N-Acetylcysteine		5619
Holo-Transferrin		2914-HT
Ala-Gln	Stable L-glutamine	5823

SMALL MOLECULES	
PRODUCT	CATALOG #
A 83-01	2939
CHIR 99021	4423
DAPT	2634
Forskolin	1099
Galunisertib	6956
Gastrin	3006
IWP 2	3533
IWP 4	5214
LDN 193189 dihydrochloride	6053
Nicotinamide	4106
SB 202190	1264
PD 0325901	4192

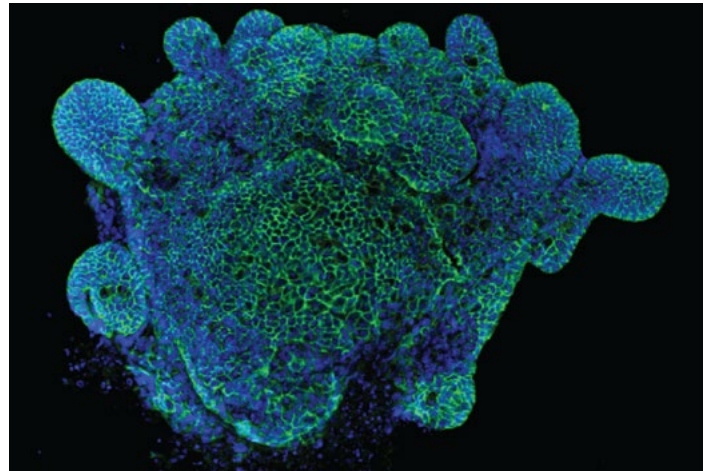
GROWTH FACTORS	
PRODUCT	CATALOG #
Recombinant Human Activin A	338-AC
Recombinant Human BMP-2	355-BM
Recombinant Human BMP-4	314-BP
Recombinant Human BMP-7	354-BP
Recombinant Human EGF	236-EG
Recombinant Human FGF-basic	233-FB
Recombinant Human FGF-7	251-KG
Recombinant Human FGF-9	273-F9
Recombinant Human FGF-10	345-FG
Recombinant Human FGF-19	969-FG
Recombinant Human HGF	294-HG
Recombinant Human Noggin	6057-NG
Recombinant Human R-Spondin 1	4645-RS
Recombinant Human R-Spondin 3	3500-RS
Recombinant Human Wnt-10b	2110-WN
Recombinant Human Wnt-3a	5036-WN

SMALL MOLECULES	
PRODUCT	CATALOG #
Prostaglandin E2	2296
Retinoic Acid	0695
SB 431542	1614
Testosterone	2822
Y-27623 dihydrochloride	1254

IMAGING ORGANOIDS

Confocal and light sheet microscopy are the recommended methods for high resolution imaging of immunostained organoids. In a Bio-Techne Virtual Organoid Symposium Q&A, members of the Hans Clevers lab referenced a 2019 Nature Protocols publication for methods of fixing and clearing organoids for 3-D imaging [Dekkers, J.F. *et al.* (2019) Nat. Protoc. 14:1756.]. The Clevers lab also published a more wholistic review of organoid imaging methods in 2018 [Rios, A. & H. Clevers (2018) Nat. Protoc. 15:24.].

R&D Systems has published protocols with tips for conserving intact organoids to analyze the expression of markers by immunostaining, which is a current challenge in the field. Additionally, the robust clarity of our new Cultrex UltiMatrix RGF Basement Membrane Extract will enable improved high-performance imaging. We also offer specific and robust primary antibodies against tissue-specific markers to further support exceptional tissue imaging and analysis. R&D Systems Organoid Resources database lists recommended tissue- and cell-specific markers for different organoid types, along with antibodies that can be used to detect these markers.



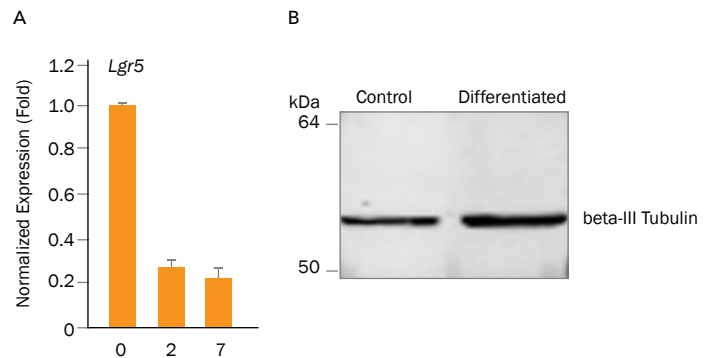
Confocal Projection Image of Mouse Intestinal Organoids. Mouse intestinal organoids were cultured using Bio-Techne reagents and processed for whole mount confocal imaging.

Learn more | rndsystems.com/organoid-recipes

CULTREX™ ORGANOID HARVESTING SOLUTION

Our catalog also includes Cultrex Organoid Harvesting Solution. This product provides a non-enzymatic method for depolymerizing extracellular matrix proteins, allowing intact organoids to be harvested for passaging, cryopreservation, or biochemical analysis.

PRODUCT	SIZE	CATALOG #
Cultrex Organoid Harvesting Solution	100 ml	3700-100-01



Cultrex Organoid Harvesting Solution is Compatible with Biochemical Analysis Techniques. Mouse small intestine organoid pellets were harvested at days 0, 2, and 7 of differentiation using [Cultrex Organoid Harvesting Solution](#) (R&D Systems, Catalog # 3700-100-01). (A) Harvested organoids were resuspended in TRIzol®, extracted for RNA, and analyzed for expression of Lgr5 using qPCR. As expected, a decrease in Lgr5 expression was detected between days 0 and 7 of differentiation. (B) Harvested organoids were resuspended in RIPA buffer for total lysate preparation. Lysates from control and differentiated organoids were analyzed by Western blot for beta-III Tubulin expression.

Learn more | rndsystems.com/organoids

ORGANOID CRYOPRESERVATION

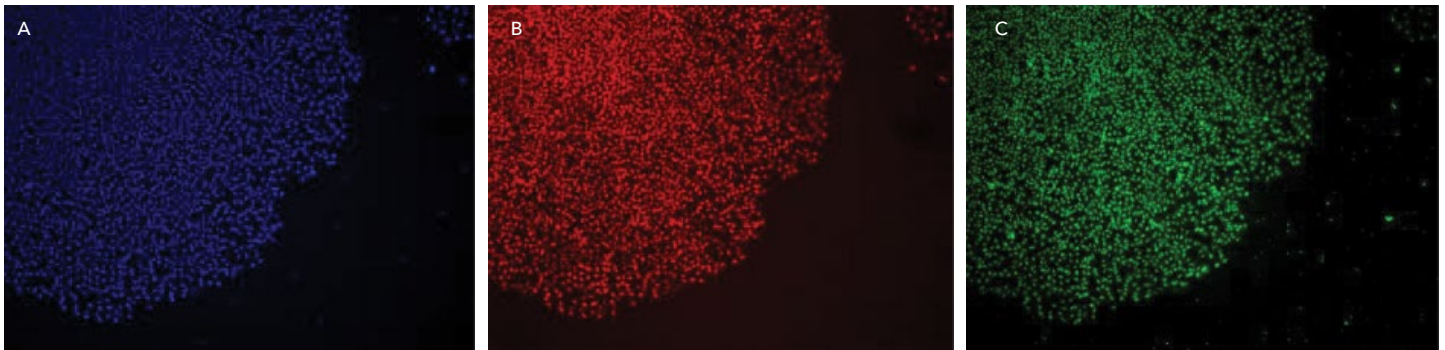
Cryopreservation of organoids is useful for cell line banking or when generating repositories of patient-derived organoids for drug discovery or toxicology testing. Similar techniques and reagents used to freeze down cell lines and primary cells can be employed for organoid cryopreservation, including base medium containing 20% FBS and 10% DMSO*. Due to their complex structural elements, troubleshooting cell viability during cryopreservation is a technical challenge. Freezing media and freeze-down strategy may need to be customized by tissue-type, organoid maturation, structure (freezing of intact structures, partially dissociated fragments, or as fully dissociated single cell suspensions), and density. R&D Systems offers basic reagents currently being used to prepare organoid cryopreservation media.

PRODUCT	CATALOG #
Fetal Bovine Serum - Premium Select	S11150
Fetal Bovine Serum - Optima	S12450
DMSO, sterile filtered	3176
*Reference organoid cryopreservation protocol based on Taniguchi Laboratory at MD Anderson Cancer Center.	

CULTREX™ STEM CELL-QUALIFIED BASEMENT MEMBRANE EXTRACT

Cultrex Stem Cell-Qualified Proteins and BME were created to help researchers choose optimal coating conditions for feeder-free culture of stem cells. Our stem cell qualified portfolio includes Cultrex RGF BME, Mouse Laminin I, Human Fibronectin, and Human Vitronectin. Each of these proteins was qualified in feeder-free cell culture to support human stem cell expansion over multiple passages.

NAME	SIZE	CATALOG #
Cultrex Stem Cell Qualified, Reduced Growth Factor Basement Membrane Extract	1 ml	3434-001-02
	5 ml	3434-005-02
	2 x 5 ml	3434-010-02

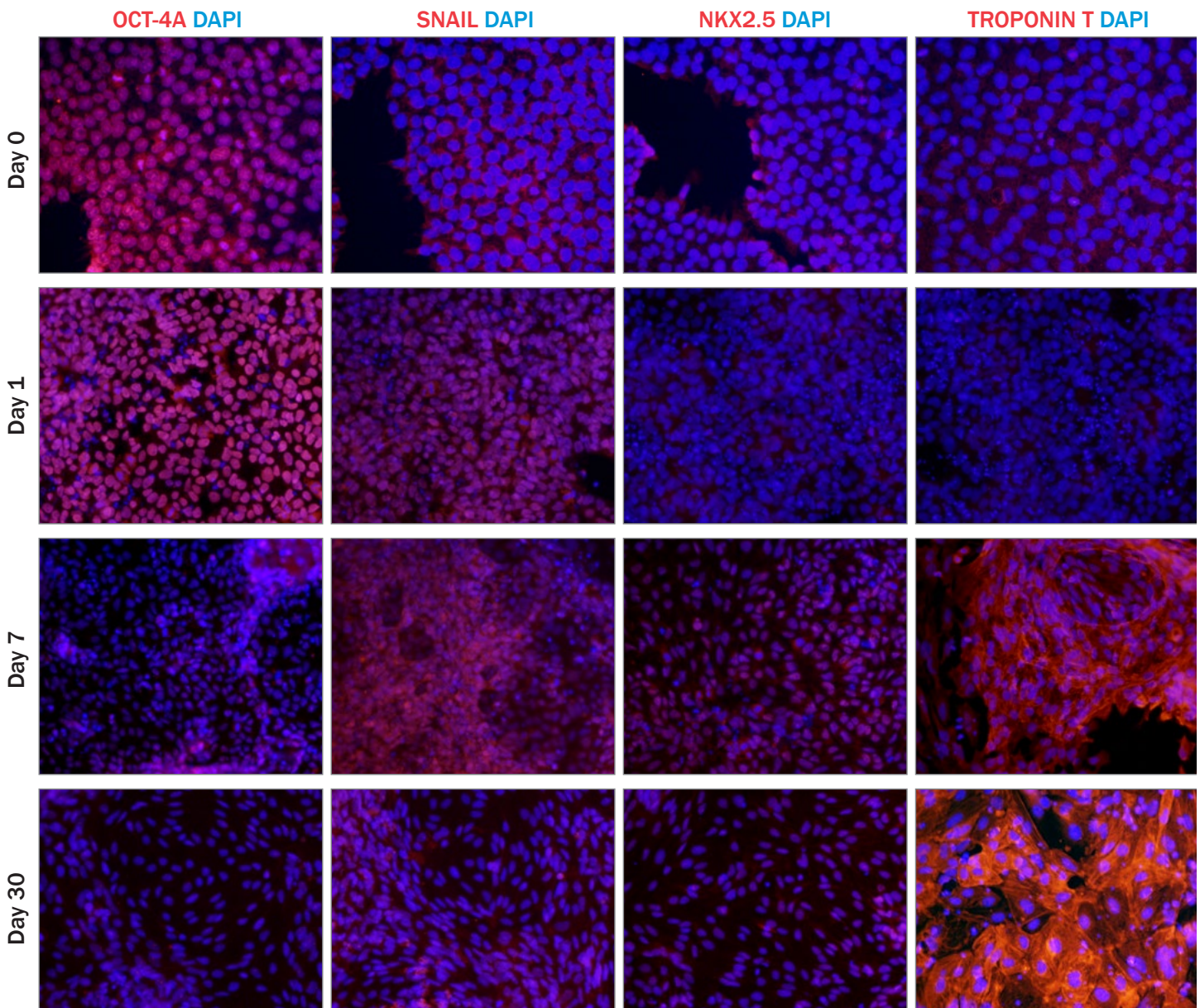


Human ESCs Maintain Stemness Markers When Cultured Using Cultrex Stem Cell Qualified BME. H9 human embryonic stem cells (ESC) were cultured using [Cultrex Stem Cell Qualified BME](#) (R&D Systems, Catalog # 3434-001-02). After four passages, these cells were assessed for stemness markers. ESCs maintain expression of the non-differentiated stem cell markers Oct-4 (B) and Nanog (C). Nuclear counterstain shown in (A) by DAPI staining. Images courtesy of the Yanik lab, MIT.

CULTREX™ STEM CELL-QUALIFIED EXTRACELLULAR MATRIX PROTEINS

NAME	SIZE	CATALOG #
Cultrex Stem Cell Qualified Mouse Laminin I	1 mg	3400-010-03
Cultrex Stem Cell Qualified Human Fibronectin, PathClear®	1 mg	3420-001-03

NAME	SIZE	CATALOG #
Cultrex Stem Cell Qualified Human Vitronectin, PathClear®	200 ug	3421-001-03



iPSC-derived Cardiomyocytes Differentiated on Cultrex Stem Cell Qualified RGF BME Express Stage-specific Markers. JOY6 human iPSCs were differentiated with the StemXVivo® Cardiomyocyte Differentiation Kit (R&D Systems, Catalog # SC032B), which features Cultrex Stem Cell-Qualified RGF BME (R&D Systems, Catalog # 3434-001-02) as a component, and assessed at select time points for stage-specific marker expression. The pluripotency marker Oct-4A is highly expressed during early differentiation (Day 0) and is subsequently downregulated. Expression of the mesoderm marker, Snail, is expressed intermediately during differentiation (Day 1). The cardiomyocyte markers NKX2.5 and Troponin T are not present in cells during early (Day 0) and intermediate (Day 1) differentiation and become more highly expressed during later stages of differentiation (Day 7, Day 30).

CULTREX™ XENOGRAFT/TUMORGRAFT PRODUCTS

Tumor xenografts are the most common models to study human cancer. The model requires the implantation of tumor-derived cells into animal hosts. In 1990, it was found that the co-injection of Cultrex™ BME with tumor cells improved tumor take and growth. We have developed, produced, and qualified Cultrex BME, Type 3, a version of BME that mimics the tumor microenvironment and is specifically designed for use in *in vivo* studies.

NAME	SIZE	CATALOG #
Cultrex Basement Membrane Extract, Type 3	1 ml	3632-001-02
	5 ml	3632-005-02
	2 x 5 ml	3632-010-02

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