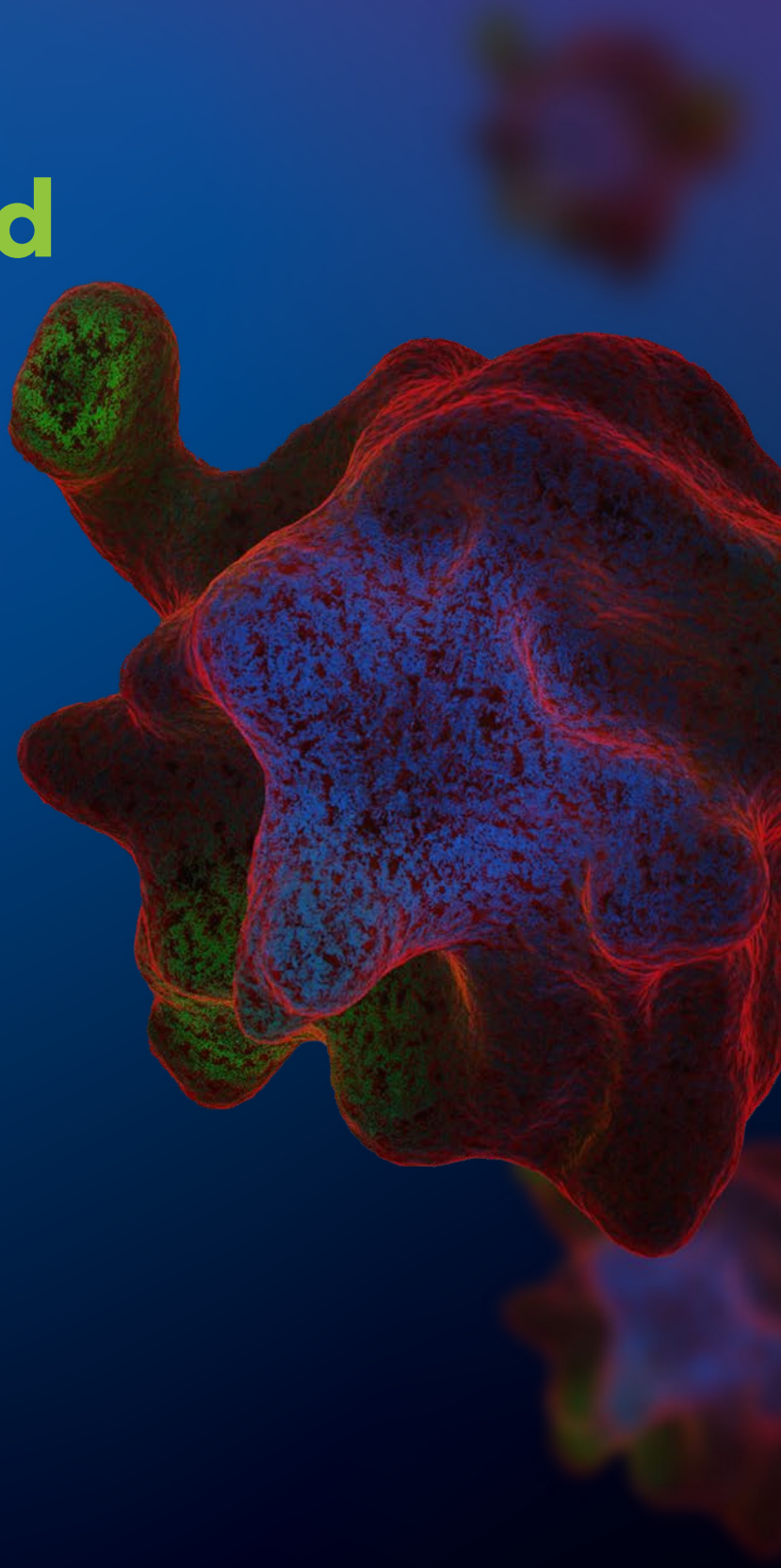
The Organoid Handbook

Building Better Organoids



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Introduction

An organoid is a miniaturized version of an organ produced *in vitro* that shows realistic micro-anatomy, is capable of self-renewal and self-organization, and exhibits similar functionality as the tissue of origin. Organoids are model systems that, in conjunction with advances in cell reprogramming technology and gene editing methods, allow unprecedented insight into human development, disease modeling, drug screening, and transplantation.

Organoids can be classified into those that are tissue-derived and those that are pluripotent stem cell-derived. Tissue-derived organoids typically originate from adult tissues while stem cell-derived organoids are established from embryonic (ESC) or induced pluripotent stem cells (iPSC). Researchers have devised methods to generate physiologically relevant organoid models for many organs, including the intestines, lung, brain, liver, pancreas, and heart. While methods for generating organoids are still evolving, presently they are providing exciting and more accurate systems that are advancing our understanding of basic organ biology and tissue regeneration.

This handbook provides a resource for key publications, protocols, reagents, and troubleshooting recommendations for organoid cell culture.

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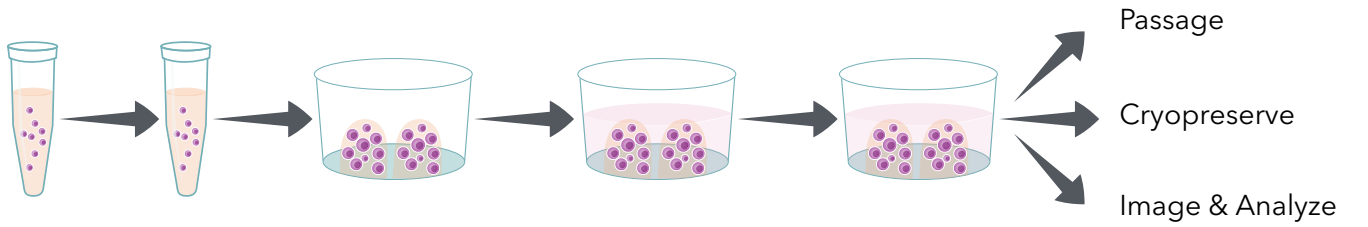
Organoid Culture

While different methods, such as the use of low adhesion round bottom dishes and bioreactors, have been employed for organoid generation, generally organoids are cultured in tissue culture plates while embedded in "domes" of purified extracellular matrix hydrogels and submerged in organoid-specific culture medium. Multiple organoids are often cultured in one "dome" and, with media changes, submerged organoids can remain in long-term culture to accommodate developmental and maturation timelines.

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 two types of Cultrex BME, Cultrex UltiMatrix Reduced Growth Factor Basement Membrane Extract and Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2. Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2, is a popular matrix used to support robust and reproducible cultures for a wide variety of organoids.

Cultrex UltiMatrix Reduced Growth Factor (RGF) Basement Membrane Extract (BME) is our newly developed, optimized organoid matrix. It 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, which make Cultrex UltiMatrix RGF BME an ideal cell scaffolding matrix for organoid cell culture, induced pluripotent stem cell expansion and differentiation, spheroid formation, and other 2-D and 3-D culture applications.



1. Isolate primary tissue stem cells or collect induced pluripotent stem cells
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 µL of the Cultrex/cell mixture. Polymerize at 37 °C for 30 minutes.
4. After polymerization, add culture medium.
5. Harvest cells by adding Organoid Cell Harvesting Buffer

Figure 1: General Schematic of Organoid Cell Culture. Individual cells or organoid fragments are embedded within a liquid extracellular matrix (ECM) and dispensed as small droplets onto the surface of a warm tissue culture plastic vessel. The ECM will solidify into a gel after incubation at 37°C and can then be covered with culture medium. Organoids will develop within the dome as 3D structures that can be harvested followed by passaging, cryopreservation, or analysis.

> ***"Our research is greatly facilitated by Bio-Techne products. The various organoid systems and co-cultures are all performed in or on R&D Systems Cultrex Reduced Growth Factor BME with great results."***
>
> Jens Puschhof, Hans Clevers lab, Hubrecht Institute, The Netherlands.

Optimize Organoid Culture Conditions with High Quality, Consistent Reagents

With the increased complexity of organoids and their culture and protocols, the risk of aberrant differentiation and culture variability can also increase. For example, the starting materials (iPSCs or adult stem cells/tissue) interaction with the extracellular matrix is critical for physiological development of the organoid. It is important that individual organoids do not come into contact with one another or with cell culture plastic as this can disrupt or advance organoid development. In addition, the quality and consistency of reagents (e.g., recombinant proteins, small molecules, and extracellular matrix hydrogels) are key elements for developing and maintaining robust and consistent organoid culture protocols.

Leading organoid researchers agree that reproducibility and culture longevity are the biggest challenges facing organoid biology. Some recommended techniques that can improve long-term culture quality and increase model consistency are: using reproducible reagents, following consistent culturing protocols (media formulations, splitting protocols, and timing), filtering organoids during passaging to facilitate consistent organoid size, and optimizing organoid density in matrices while ensuring tissue remains fully embedded and does not contact cell culture plastic.

> ***One of the key features of organoids in general and those used in this study is the defined media that we use. This allows us to tailor the in vitro niche environment to the specific cells we are growing or to the cells that we are trying to generate in culture. This has been made possible, in part, through the use of a wide range of different growth factors and small molecules from R&D Systems and Tocris. We also used Cultrex RGF BME for all of the organoids in this study.***
>
> Dr. Talya Dayton, Hubrecht Institute, The Netherlands.

> ***Ultimatrix is unique in its ability to bead up to form a droplet at even low concentrations. We have found that preparing protein concentrations below 8 mg/mL for other commercial ECMs causes 3-dimensional tissues, such as intestinal organoids, to sink to the plastic leading to differentiation and cell death, while UltiMatrix supports normal growth.***
>
> Michael K. Dame, Associate Director, Translational Tissue Modeling Laboratory, Jason Spence Laboratory, University of Michigan, MI, USA

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Application Note | Optimizing Organoid Culture Conditions: The Importance of Growth Factor Bioactivity and Reagent Consistency

Intestinal Organoids

The small intestine, large intestine, and colon consist of a multicellular epithelium with distinct morphological structures, including villi and invaginated crypt structures. Intestinal crypts house Lgr5+ intestinal adult stem cells that are responsible for the continuous renewal of intestinal epithelium and were first utilized to create long-term 3D culture models of the intestine, termed intestinal organoids or epithelial organoids. These organoid cultures are employed to study normal and diseased physiology, including barrier functions, nutrient uptake, and tissue renewal. In addition, intestinal organoids can be generated from iPSCs. iPSC-derived organoids have been used as advanced models for gastrointestinal developmental biology, drug toxicity, and personalized medicine applications.

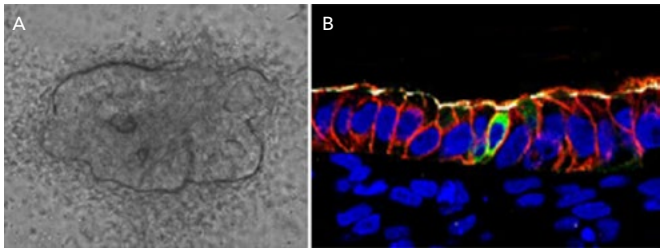


Figure 2. iPSC-derived Intestinal Organoids Grown in Cultrex UltiMatrix RGF Basement Membrane Extract. Human iPSCs were embedded in Cultrex UltiMatrix RGF Basement Membrane Extract (R&D Systems, Catalog # BME001-05) and cultured in growth medium. hiPSC-derived intestinal organoids cultured for 13 days were imaged using (A) brightfield microscopy or processed and (B) stained for Chromagranin A (green), Villin (white), E-Cadherin (red), and DAPI (blue).

Reagents Used for Intestinal Organoid Culture

Product Name	Supplier	Catalog #
Cultrex UltiMatrix Reduced Growth Factor Basement or Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2	R&D Systems	BME001-05 3533-005-02
Advanced DMEM/F-12 Cell Culture Medium	Thermo Fisher	12634-010
GlutaminePlus	R&D Systems	B90210
Penicillin/Streptomycin	R&D Systems	B21210
HEPES	Tocris Bioscience	R35150
N21-MAX Supplement	R&D Systems	AR008
N-Acetylcysteine	Tocris Bioscience	5619
Y-27632 dihydrochloride (Rho Kinase inhibitor)	Tocris Bioscience	1254

Product Name	Supplier	Catalog #
Nicotinamide	Tocris Bioscience	4106
SB 202190 (p38 MAPK inhibitor)	Tocris Bioscience	1264
Prostaglandin E2 (PGE2)	Tocris Bioscience	2296
A 83-01 (ALK5 inhibitor)	Tocris Bioscience	2939
Recombinant Human EGF	R&D Systems	236-EG
Recombinant Human R-Spondin 1	R&D Systems	4645-RS
Recombinant Human Noggin	R&D Systems	6057-NG
Recombinant Human Wnt-3a	R&D Systems	5036-WN
Cultrex Organoid Harvesting Solution	R&D Systems	3700-100-01

Notable Publications and Protocols

Publication	Description	Bio-Techne Reagents Used	Catalog #
Mahe M. *et al.* (2013) Curr. Protoc. Mouse Biol. **3**:217.	Establishment of gastrointestinal epithelial organoids.	Recombinant Mouse Noggin	6997-NG
		Recombinant Mouse Wnt-3a	1324-WN
		Recombinant Human EGF	236-EG
		Recombinant Human Jagged 1 Fc Chimera Protein	1277-JG
Co, J.Y. *et al.* (2019) Cell Reports **26**:2509.	Human enteroid model for host-pathogen interactions.	Cultrex RGF Basement Membrane Extract, Type 2	3533-005-02
		A 83-01	2939
		CHIR 99021 (GSK-3 inhibitor)	4423

View the complete Human Intestinal Organoid Culture protocol available in our Organoid Resources Database

Gastric Organoids

Similar to the intestine, the stomach contains Lgr5+ adult stem cells that can be isolated, cultured, and differentiated *in vitro* into gastric organoids. Early organoid models elucidated molecular mechanisms underlying gastric development, including signaling pathways that influence fundic or antral gastric epithelium formation. Gastric organoid cultures are powerful models to study normal and diseased gastric physiology as well as more complex models for drug discovery and disease modeling.

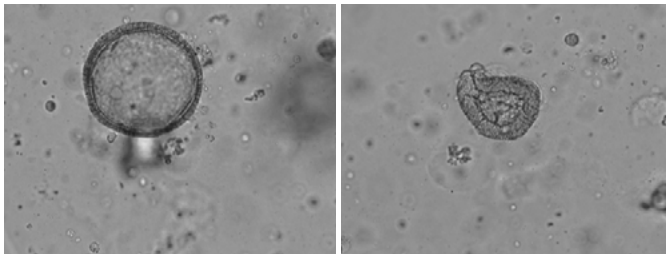


Figure 3. Undifferentiated Human Gastric Organoids. Representative brightfield images of human gastric organoids that were cultured using Cultrex RGF BME, Type 2 (R&D Systems, Catalog # 3533-005-02) and the Bio-Techne reagents listed in this protocol.

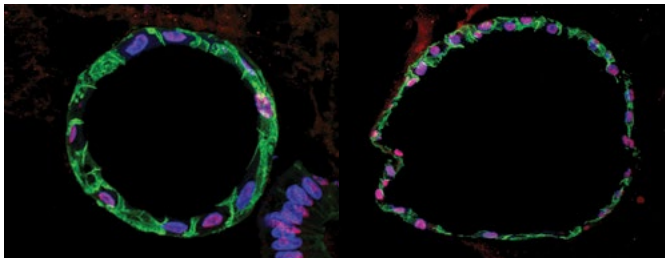


Figure 4. Immunohistochemistry of Undifferentiated Human Gastric Organoids. Human gastric organoids were cultured using Cultrex RGF BME, Type 2 and the Bio-Techne reagents listed in this protocol. Undifferentiated colon organoids were stained using the Human/Mouse E-Cadherin Antibody (green; R&D Systems; Catalog # AF748), the Human HOXB7 Antibody (red; R&D Systems; Catalog # MAB8040), and counterstained with DAPI (blue; R&D Systems; Catalog # 5748).

Reagents Used for Gastric Organoid Culture

Product Name	Supplier	Catalog #
Cultrex Organoid Harvesting Solution	R&D Systems	3700-100-01
Cultrex UltiMatrix Reduced Growth Factor Basement Membrane Extract or Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2	R&D Systems	BME001-05 3533-005-02
Advanced DMEM/F-12 Cell Culture Medium	Thermo Fisher	12634-010
GlutaminePlus	R&D Systems	B90210
HEPES	Tocris Bioscience	3173
N21-MAX Supplement	R&D Systems	AR008
N-2 MAX Supplement	R&D Systems	AR009
N-Acetylcysteine	Tocris Bioscience	5619
Gastrin I (Human)	Tocris Bioscience	3006

Product Name	Supplier	Catalog #
SB 202190 (p38 MAPK Inhibitor)	Tocris Bioscience	1264
Nicotinamide	Tocris Bioscience	4106
Human Insulin, Solution	Sigma-Aldrich	I9278
Human Transferrin	Sigma-Aldrich	T8158
Y-27632 dihydrochloride (Rho Kinase Inhibitor)	Tocris Bioscience	1254
Recombinant Human EGF	R&D Systems	236-EG
Recombinant Human R-Spondin 1	R&D Systems	4645-RS
Recombinant Human Noggin	R&D Systems	6057-NG
Recombinant Human FGF-10	R&D Systems	345-FG
Recombinant Human Wnt-3a	R&D Systems	5036-WN
A 83-01 (ALK5 inhibitor)	Tocris Bioscience	2939
CHIR 99021 (GSK-3 inhibitor)	Tocris Bioscience	4423

View the complete Human Gastric Organoid Culture protocol available in our Organoid Resources Database

Notable Publications and Protocols

Publication	Description	Bio-Techne Reagents Used	Catalog #
McCracken, E.M. *et al.* (2014) Nature **516**:400. McCracken, E.M. *et al.* (2017) Nature **541**:182.	Modelling human development and disease in pluripotent stem-cell-derived gastric organoids.	Recombinant Human BMP-4	314-BP
		Recombinant Human FGF-4	235-F4
		Recombinant Human Noggin	6057-NG
		Recombinant Human EGF	236-EG
		Recombinant Human FGF-10	345-FG
		Recombinant Human Wnt-5a	645-WN
Munero J. *et al.* (2017) Cell Stem Cell **21**:51.	iPSC differentiation into colonic organoids.	Y-27632 dihydrochloride	1254
		Recombinant Human FGF-4	235-F4
		Recombinant Human BMP-2	355-BM
		Recombinant Human EGF	236-EG
		Recombinant Human Noggin	6057-NG
		CHIR 99021	4423
		SAG	4366
Li, X. *et al.* (2018) Nature Comm. **9**:2983.	Organoid cultures provide a model for recapitulate esophageal adeno carcinoma.	Cultrex RGF Basement Membrane Extract, Type 2	3533-005-02
		Cultrex HA-R-Spondin1-Fc 293T Cells	3710-001-01
		A 83-01	2939

Liver Organoids

The liver is the primary organ system for drug metabolism and detoxification. In this role, it is also highly susceptible to damage from pharmaceuticals and other chemical toxicants. Animal models and traditional *in vitro* assays modeling liver metabolism often fail to recapitulate the *in vivo* toxicity of drugs in human patients. Liver organoids, derived from primary tissue or induced pluripotent stem cells, have emerged as more complex and predictive models for hepatotoxicity and drug screening.

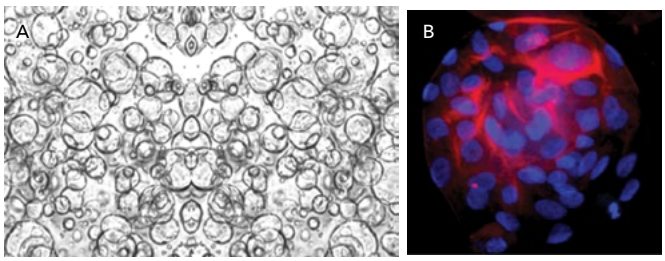


Figure 5. Human Liver Organoids. A) Brightfield Image of human undifferentiated liver organoids cultured using Cultrex UltiMatrix RGF Basement Membrane Extract (R&D Systems, Catalog # BME001-05) and in media featuring Bio-Techne reagents. **B**) Expression of Albumin (red; R&D Systems, Catalog # MAB1455) in differentiated human liver organoids. Image counterstained with DAPI (blue; Tocris, Catalog # 5748).

Reagents Used For Liver Organoid Culture

Product Name	Supplier	Catalog #
Cultrex Organoid Harvesting Solution	R&D Systems	3700-100-01
Cultrex UltiMatrix Reduced Growth Factor Basement Membrane Extract or Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2	R&D Systems	BME001-05 3533-005-02
GlutaminePlus	R&D Systems	B90210
HEPES	Tocris Bioscience	3173
N21-MAX Supplement	R&D Systems	AR008
N-2 MAX Supplement	R&D Systems	AR009
N-Acetylcysteine	Tocris Bioscience	5619
Gastrin I (Human)	Tocris Bioscience	3006
Nicotinamide	Tocris Bioscience	4106
Y-27632 dihydrochloride (Rho Kinase inhibitor)	Tocris Bioscience	1254

Product Name	Supplier	Catalog #
Recombinant Human EGF	R&D Systems	236-EG
Recombinant Human R-Spondin 1	R&D Systems	4645-RS
Recombinant Human Noggin	R&D Systems	6057-NG
Recombinant Human FGF-10	R&D Systems	345-FG
Recombinant Human FGF-19	R&D Systems	969-FG
Recombinant Human BMP-7	R&D Systems	354-BP
Recombinant Human HGF	R&D Systems	294-HG
Recombinant Human Wnt-3a	R&D Systems	5036-WN
Forskolin	Tocris Bioscience	1099
A 83-01 (ALK5 inhibitor)	Tocris Bioscience	2939
DAPT	Tocris Bioscience	2634
Dexamethasone	Tocris Bioscience	1126

View the complete Human Liver Organoid Culture protocol available in our Organoid Resources Database

Notable Publications and Protocols

Publication	Description	Bio-Techne Reagents Used	Catalog #
Huch, M. *et al.* (2015) Cell **160**:299.	Long-term culture of adult human liver stem cells.	Cultrex RGF Basement Membrane Extract, Type 2	3533-010-02
		A 83-01	2939
		Forskolin	1099
Ogawa, M. *et al.* (2015) Nat. Biotechnol. **33**:853.	Human iPSC-derived cholangiocyte organoids.	L-685, 458	2627
		Recombinant Human HGF	294-HG
		Recombinant Human EGF	236-EG
		Recombinant Human TGF-β1	240-B
Takebe, T. *et al.* (2013) Nature **499**:481.	Vascularized human iPSC-derived hepatic organoids.	Recombinant Human Oncostatin M (OSM)	295-OM
Broutier, L. *et al.* (2016) Nature Protocols **11**:1724.	Protocol for generating human and mouse adult liver organoids.	Cultrex RGF Basement Membrane Extract, Type 2	3533-005-02
Koike, H. *et al.* (2019) Nature **574**:112.	Modelling hepato-biliary-pancreatic organogenesis.		

Lung Organoids

3D cell culture models of the pulmonary system are increasingly utilized to study lung regeneration, model disease (i.e. cystic fibrosis), and investigate mechanisms of viral lung infection (i.e. SARS-CoV-2). While lung organoids were first generated using Lgr5+ stem cells isolated from primary tissue, protocols for culturing iPSC-derived lung organoids have increased the flexibility and accessibility of this model system for use in personalized medicine and drug discovery.

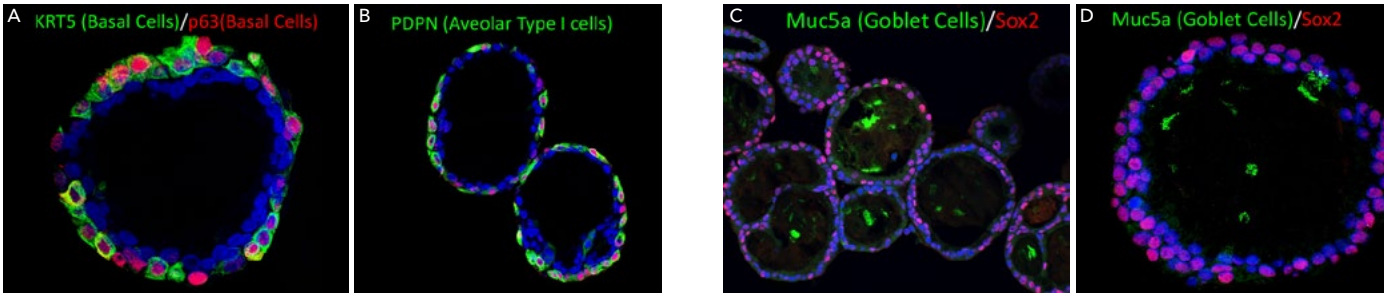


Figure 6. Human Lung Organoids. Adult stem cells isolated from human lung biopsy tissue were embedded in Cultrex UltiMatrix RGF Basement Membrane Extract (R&D Systems, Catalog # BME001-05) and cultured in media for 20-60 days. Lung organoids were able to differentiate and exhibit markers for various cell types of the lung. **A**) Lung organoids were stained with anti-Cytokeratin 5 (KRT5) (green; Novus Biologicals, Catalog # NB110-56916) and with anti-p63/TP73L (red; R&D Systems, Catalog # AF1916) to visualize basal cells. **B**) Lung organoids were stained with anti-Podoplanin (PDPN) (green; Novus Biologicals, Catalog # NB600-1015) to visualize alveolar type I cells. **C,D**) Lung organoids were stained with anti-Muc5ac (green; Novus Biologicals, Catalog # NBP2-15196) to visualize Goblet cells and for Sox2 (red; R&D Systems, Catalog # MAB2018). All samples were counterstained with the nuclear stain DAPI (blue; Tocris, Catalog # 5748).

Reagents Used For Lung Organoid Culture

Product Name	Supplier	Catalog #
Cultrex Organoid Harvesting Solution	R&D Systems	3700-100-01
Cultrex UltiMatrix Reduced Growth Factor Basement Membrane Extract or Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2	R&D Systems	BME001-05 3533-005-02
Advanced DMEM/F-12 Cell Culture Medium	Thermo Fisher	12634-010
GlutaminePlus	R&D Systems	B90210
HEPES	Tocris Bioscience	3173
N21-MAX Supplement	R&D Systems	AR008
A 83-01 (ALK5 inhibitor)	Tocris Bioscience	2939

Product Name	Supplier	Catalog #
N-Acetylcysteine	Tocris Bioscience	5619
Penicillin/Streptomycin	R&D Systems	B21210
SB 202190 (p38 MAPK inhibitor)	Tocris Bioscience	1264
Nicotinamide	Tocris Bioscience	4106
Y-27632 dihydrochloride (Rho Kinase inhibitor)	Tocris Bioscience	1254
Recombinant Human R-Spondin 1	R&D Systems	4645-RS
Recombinant Human Noggin	R&D Systems	6057-NG
Recombinant Human FGF-10	R&D Systems	345-FG
Recombinant Human FGF-7	R&D Systems	251-KG

View the complete Human Lung Organoid Culture protocol available in our Organoid Resources Database

Notable Publications and Protocols

Publication	Description	Bio-Techne Reagents Used	Catalog #
Sachs, N. *et al.* (2019) EMBO J. **34**:e100300.		Cultrex RGF Basement Membrane Extract, Type 2	3533-005-02
Miller, A. J. *et al.* (2019) Nature Protocols **14**:518.	Protocol for generating human iPSC-derived lung organoids.	Recombinant Human Activin A	338-AC
		Recombinant Human Noggin	6057-NG
		Recombinant Human FGF-10	345-FG
		Recombinant Human FGF-4	7460-F4
		Recombinant Human FGF-7	251-KG
Dye, B.R. *et al.* (2015) eLife **4**:e05098.	Protocol for generating human iPSC-derived lung organoids.	Recombinant Human Activin A	338-AC
		Recombinant Human Noggin	6057-NG
		Recombinant Human FGF-2	233-FB
		Recombinant Human FGF-4	7460-F4
		Recombinant Human Sonic Hedgehog	8908-SH

Brain Organoids

Protocols to generate 3D brain organoids from ESCs and iPSCs were first published in 2009. These studies showed that pluripotent stem cells could differentiate into cerebral organoids containing specific cortical regions, neural progenitor populations, and cortical layer patterning. Cerebral organoids have since been employed to uncover evolutionary differences in brain development between species, mechanisms of brain region interconnectivity, and the developmental physiology of normal and diseased brain regions. iPSC-derived organoids show great potential for use in drug discovery as well as modeling neurodegenerative disease and viral brain infection.

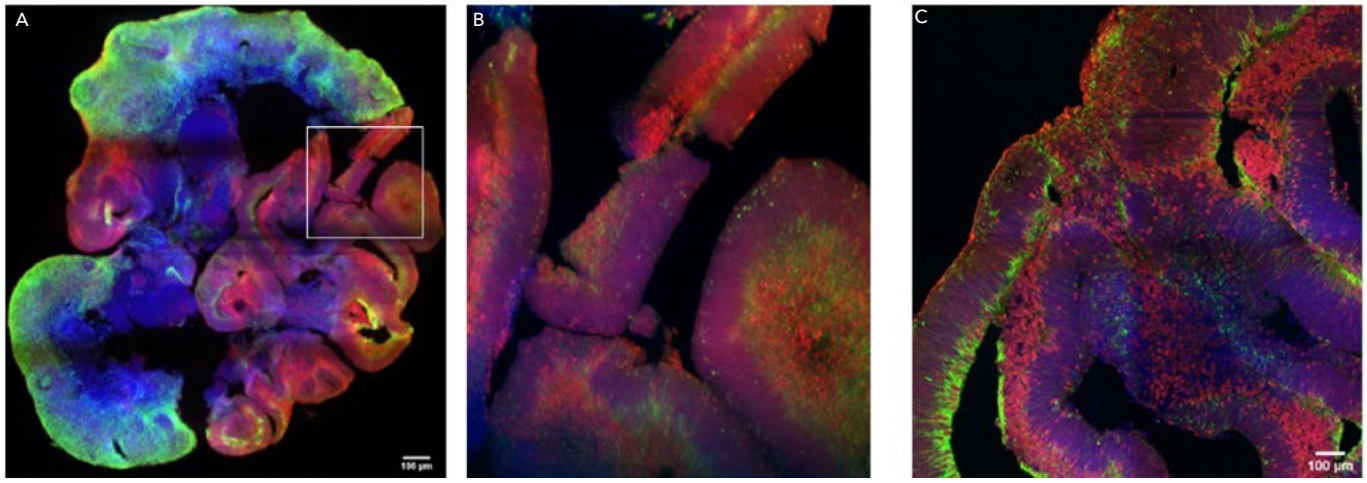


Figure 7. Cerebral Organoids Grown in Cultrex UltiMatrix RGF Basement Membrane Extract. iPSC-derived cerebral organoids (day 45) were cultured using Cultrex UltiMatrix RGF Basement Membrane Extract (R&D Systems, Catalog # BME001-05) and stained for Syto16 (blue), Pax6 (red), and Vimentin (green). **A**) Image taken at 4x magnification. **B**) An enlarged view of the area shown within the white box in part A of the figure. **C**) Image taken at 15x magnification. Images courtesy of LifeCanvas Technologies.

Reagents Used For Brain Organoid Culture

Product Name	Supplier	Catalog #
Cultrex Organoid Harvesting Solution	R&D Systems	3700-100-01
Cultrex UltiMatrix Reduced Growth Factor Basement Membrane Extract or Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2	R&D Systems	BME001-05 3533-005-02
Advanced DMEM/F-12 Cell Culture Medium	Thermo Fisher	12634-010
N-2 MAX Supplement	R&D Systems	AR009
N21-MAX Supplement	R&D Systems	AR008

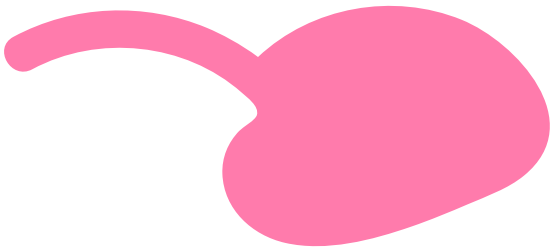
Product Name	Supplier	Catalog #
N21-MAX Vitamin A Free Supplement	R&D Systems	AR012
Penicillin/Streptomycin	R&D Systems	B21210
GlutaminePlus	R&D Systems	B90210
Insulin		
2-mercaptoethanol		
Recombinant Human FGF basic	R&D Systems	3718-FB
Recombinant Human Noggin	R&D Systems	6057-NG
Y-27632 dihydrochloride (Rho Kinase inhibitor)	Tocris Bioscience	1254

Notable Publications and Protocols

Publication	Description	Bio-Techne Reagents Used	Catalog #
Pollen, A.A. *et al.* (2019) Cell **176**:743.	Establishing cerebral organoids as models of human-specific brain evolution	Y-27632 dihydrochloride	1254
		SB 431542	1614
Bershteyn, M. *et al.* (2017) Cell Stem Cell **20**:435.	Human iPSC-derived cerebral organoids model cellular features of lissencephaly and reveal prolonged mitosis of outer radial glia	Y-27632 dihydrochloride	1254
		SB 431542	1614
Bagley, J. A. *et al.* (2017) Nat. Methods **13**:743.	Fused cerebral organoids model interactions between brain regions		
Lancaster M.A. and J. A. Knoblich (2014) Nat. Protocols **9**:2329.	Generation of cerebral organoids from human pluripotent stem cells		

Kidney Organoids

Using pluripotent stem cells, kidney organoid culturing protocols have shown the ability to recapitulate the organ's complex tissue cytoarchitecture, including expression of cellular markers for podocytes, proximal tubules, and distal tubules. Success in cultivating kidney organoids has facilitated research interrogating kidney development, physiology, and mechanisms underlying kidney disease (i.e. chronic kidney disease). In addition, kidney organoid research has demonstrated its potential as a translational method for kidney tissue regeneration.



Reagents Used For Kidney Organoid Culture

Product Name	Supplier	Catalog #
Cultrex Organoid Harvesting Solution	R&D Systems	3700-100-01
Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2	R&D Systems	3533-005-02
Advanced DMEM/F-12 Cell Culture Medium	Thermo Fisher	12634-010
N-2 MAX Supplement	R&D Systems	AR009
N21-MAX Supplement	R&D Systems	AR008
Penicillin/Streptomycin	R&D Systems	B21210
N-Acetylcysteine	Tocris Bioscience	5619
GlutaminePlus	R&D Systems	B90210
Holo-Transferrin		

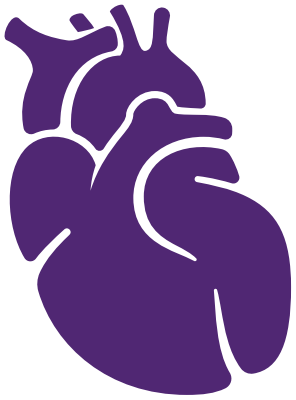
Product Name	Supplier	Catalog #
Advanced DMEM/F-12	Thermo Fisher	12634-010
Recombinant Human Activin A	R&D Systems	338-AC
Recombinant Human BMP-2	R&D Systems	355-BM
Recombinant Human BMP-4	R&D Systems	314-BP
Recombinant Human FGF basic	R&D Systems	3718-FB
Recombinant Human FGF-9	R&D Systems	273-F9
CHIR 99021 (GSK-3 inhibitor)	Tocris Bioscience	4423
Retinoic Acid	Tocris Bioscience	695
Y-27632 dihydrochloride (Rho Kinase inhibitor)	Tocris Bioscience	1254

Notable Publications and Protocols

Publication	Description	Bio-Techne Reagents Used	Catalog #
Taguchi, A. *et al.* (2014) Cell Stem Cell **14**:53.	iPSC-nephron progenitor role in developing kidney organoids.	Recombinant Human Activin A	338-AC
		Recombinant Human BMP-4	314-BP
		Recombinant Human FGF-2	233-FB
		Recombinant Human FGF-9	273-F9
Morizane, R. *et al.* (2015) Nat. Biotechnol. **33**:1193.	Nephron organoids derived from human pluripotent stem cells.	Recombinant Human Activin A	338-AC
		Recombinant Human FGF-9	273-F9
		Y-27632 dihydrochloride	1254
		CHIR 99021	4423
Freedman, B.S. *et al.* (2015) Nat. Comm. **6**:8715.	Gene editing of kidney organoids to model disease.	IWP 2	3533
Takasato, M. *et al.* (2015) Nature **526**:564.	Human iPSC-derived kidney organoid generation.		

Heart Organoids

In vitro generation of cardiac tissue is enabling advancements in drug discovery and toxicity testing, as well as facilitating the engineering of cardiac tissue for regenerative therapies. Various methods have been employed to generate 3D cardiac tissue, including iPSC-derived cardiomyocyte spheroids and bioprinting of cardiac organoids with iPSCs that are subsequently differentiated into cardiomyocytes. However, protocol and reagent advancements are still needed to enhance the maturity and complexity of the cardiac tissue.



Notable Publications and Protocols

Publication	Description	Bio-Techne Reagents Used	Catalog #
Andersen, P. (2018) Nat. Comm. **9**:3140.	Precardiac spheroids generated from human pluripotent stem cells.	Recombinant Human Activin A	338-AC
		Recombinant Human BMP-4	314-BP
		Recombinant Human Wnt-3a	5036-WN
		Recombinant Human Wnt-5a	645-WN
		Recombinant Human Wnt-11	6179-WN
Kupfer, M.E. *et al.* (2020) Circulation Research **127**:207.	Cardiac organoid formation using different extracellular matrix proteins.		
Mills, R.J. *et al.* (2017) PNAS **113**:E8372.	Cardiac organoids from human iPSCs		

Mammary Organoids

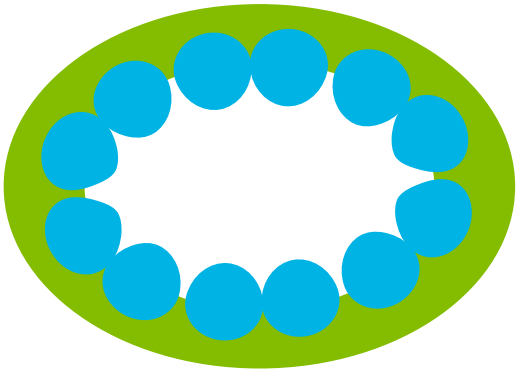
Protocols to generate mammary organoids from primary epithelial tissues are helping elucidate the cell fate decisions and molecular mechanisms of mammary gland development, including ductal formation and transformation of milk-producing alveoli. Most importantly, these 3D culture techniques have enabled the cultivation of a breast cancer organoids, which are being employed for *in vitro* drug discovery and personalized drug screening for breast cancer.

Notable Publications and Protocols

Publication	Description	Bio-Techne Reagents Used	Catalog #
Sachs, N. *et al.* (2018) Cell **172**:373.	Robust protocol for long-term culturing of human mammary epithelial organoids.	Cultrex RGF Basement Membrane Extract, Type 2	3533-005-02
		Recombinant Human R-Spondin 3	3500-RS
Rosenbluth, J.M. (2020) Nat. Commun. **11**: 1711.	Human mammary organoids derived from breast tissue.	A 83-01	2939
Jamieson, P. *et al.* (2017) Stem Cells and Regeneration **144**:1065.	Mouse mammary organoids derived from epithelial cells.	Cultrex RGF Basement Membrane Extract, Type 2	3533-005-02
		Y-27632 dihydrochloride	1254

Pancreatic Organoids

Pancreatic organoids have become an informative *in vitro* model to study pancreatic cancer, exocrine disease, and the basic development of pancreatic ductal epithelium for potential use as regenerative or therapeutic treatment of diabetes. While robust protocols for pancreatic organoid generation using mouse primary pancreatic ductal tissues exist, protocols that support the long-term cultivation of pancreatic organoids from human tissues are still emerging.



Reagents Used For Pancreatic Organoid Culture

Product Name	Supplier	Catalog #
Cultrex Organoid Harvesting Solution	R&D Systems	3700-100-01
Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2	R&D Systems	3533-005-02
N-2 MAX Supplement	R&D Systems	AR009
N21-MAX Supplement	R&D Systems	AR008
N-Acetylcysteine	Tocris Bioscience	5619
Penicillin/Streptomycin	R&D Systems	B21210
GlutaminePlus	R&D Systems	B90210
Advanced DMEM/F-12	Thermo Fisher	12634-010

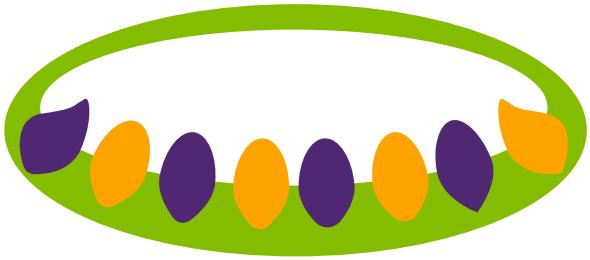
Product Name	Supplier	Catalog #
Recombinant Human EGF	R&D Systems	236-EG
Recombinant Human FGF-10	R&D Systems	345-FG
Recombinant Human Noggin	R&D Systems	6057-NG
Recombinant Human R-Spondin 1	R&D Systems	4645-RS
Recombinant Human Wnt-3a	R&D Systems	5036-WN
A 83-01 (ALK5 inhibitor)	Tocris Bioscience	2939
Nicotinamide	Tocris Bioscience	4106
Gastrin I (Human)	Tocris Bioscience	3006

Notable Publications and Protocols

Publication	Description	Bio-Techne Reagents Used	Catalog #
Georgakopoulos, N. *et al.* (2020) BMC Developmental Biology **20**:4.	Long-term expansion of adult human pancreatic organoids.	Cultrex RGF Basement Membrane Extract, Type 2	3533-005-002
		PGE-2	2296
		Forskolin	1099
		A 83-01	2939
Broutier, L. *et al.* (2016) Nat. Protocols **11**:1724.	Protocol to culture self-renewing human pancreatic organoids.	Cultrex RGF Basement Membrane Extract, Type 2	3533-005-02
		Cultrex HA-R-Spondin 1-Fc 293T Cells	3710-001-01
		Recombinant Human FGF-19	969-FG
		A 83-01	2939
		PGE-2	2296
Greggio, C. *et al.* (2013) Development **140**:4452.	Effects of reagents and matrices on pancreatic organoid culture.	Recombinant Human R-Spondin 1	4645-RS
		Recombinant Human FGF-2	233-FB
		Recombinant Human FGF-10	345-FG
Dossena, M. *et al.* (2020) Stem Cell Research & Therapy **11**:94.	GMP-compliant culture of human pancreatic organoids.	Recombinant Human R-Spondin 1	4645-RS
		A 83-01	2939
		PGE-2	2296

Inner Ear Organoids

Pluripotent stem cell-derived inner ear organoids are rapidly advancing our understanding of inner ear development and physiology. Inner ear organoids have been shown to develop sensory epithelium containing the necessary hair cells, supporting cells, and synaptic-like structures that support auditory or gravitational transduction. These models have great potential for translational research, uncovering molecular and cellular mechanisms that support the regeneration of cochlear and vestibular sensory tissue.



Reagents Used For Inner Ear Organoid Culture

Product Name	Supplier	Catalog #
Cultrex Organoid Harvesting Solution	R&D Systems	3700-100-01
Cultrex Reduced Growth Factor Basement Membrane Extract, Type 2	R&D Systems	3533-005-02
N-2 MAX Supplement	R&D Systems	AR009
N21-MAX Supplement	R&D Systems	AR008
Recombinant Human Leukemia Inhibitor Factor (LIF)	R&D Systems	7734-LF
CHIR 99021 (GSK-3 inhibitor)	Tocris Bioscience	4423
PD 0325901 (MEK inhibitor)	Tocris Bioscience	4192
Penicillin/Streptomycin	R&D Systems	B21210
GlutaminePlus	R&D Systems	B90210
Advanced DMEM/F-12	Thermo Fisher	12634-010
Recombinant Human BMP-4	R&D Systems	314-BP
Recombinant Human FGF basic	R&D Systems	3718-FB
A 83-01 (ALK5 inhibitor)	Tocris Bioscience	2939
SB 431542	Tocris Bioscience	1614

Organoid Harvesting

Organoids are often cultured in matrix hydrogels that promote the growth of 3D structures but also must be removed before passaging, cryopreservation, and analysis of the organoids. Proteases can be employed to degrade the extracellular proteins within the organoid matrix. However, non-enzymatic methods of matrix depolymerization, such as Cultrex Organoid Harvesting Solution, are preferred because they limit carryover of protease activity in subsequent cultures or product analysis.

Product Name	Supplier	Catalog #
Cultrex Organoid Harvesting Solution	R&D Systems	3700-100-01

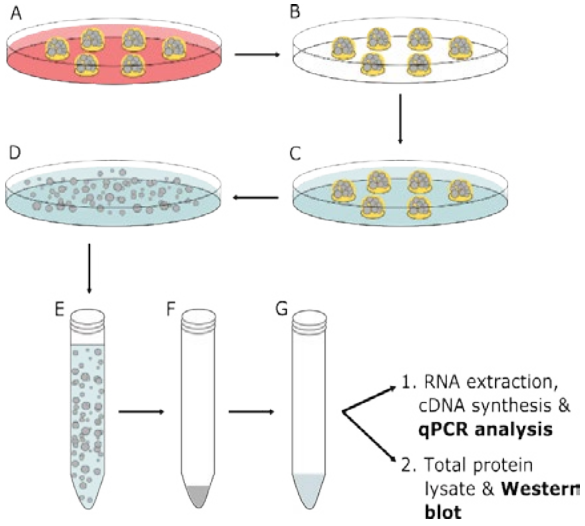


Figure 8. Summarized Protocol to Harvest Organoids for Biochemical Analysis. A) Treat organoids with differentiation medium. **B**) Discard medium. **C**) Add Cultrex Organoid Harvesting Solution. **D**) Incubate at 2–8 °C. **E**) Transfer organoids to a conical tube. **F**) Centrifuge organoids. **G**) Resuspend organoids in the appropriate lysis solution for either RNA extraction or protein analysis.

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 Name	Supplier	Catalog #
Fetal Bovine Serum - Premium Select	R&D Systems	S11150
Fetal Bovine Serum - Optima	R&D Systems	S12450
DMSO, sterile filtered	Tocris	3176

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 reference a 2019 Nature Protocols publication for methods of fixing and clearing organoids for 3D imaging (Dekkers, J.F. et al. (2019) Nature Protocols 14:1756). Rios and Clevers also published a more wholistic review of organoid imaging methods: Rios, A. and H. Clevers (2018) Nature Protocols 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. In addition to retaining tissue integrity and ensuring matrix clarity for imaging, choosing robust and specific primary antibodies against tissue-specific markers will benefit tissue imaging and analysis. R&D Systems Organoid Resource Guide provides a list of primary antibodies against common tissue- and cell-specific markers within different organoid types.

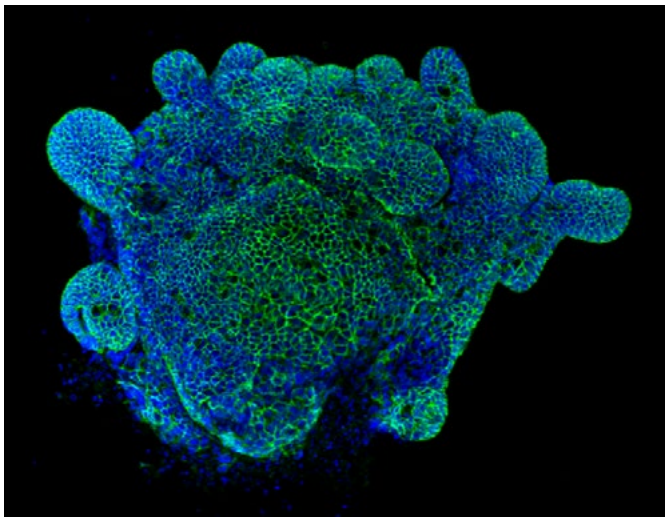


Figure 9. Confocal Projection Image of Mouse Intestinal Organoids. Mouse intestinal organoids were cultured using Bio-Techne reagents and processed for whole mount confocal imaging.

Single Cell *In Situ* Hybridization in Organoids

In situ hybridization (ISH) in organoids enables researchers to visualize RNA expression and distribution at the single cell level. RNAScope™ ISH is the leading technology for the quick and precise cell-specific localization of RNA transcripts and is being employed globally by organoid researchers.

RNAScope is being used to identify, characterize and locate stem cell populations and detect stem cell and tissue-specific markers when no reliable antibodies are available.

Learn more | bio-techne.com/reagents/rnascope-ish-technology

> ***Despite limited experience with in situ hybridization, we were able to visualize LGR5 and WDR43 with relative ease using probes and reagents from ACD.***
>
> Dr Robert Barrett, Cedars-Sinai Medical Center, CA, USA.

Organoid Viability

Monitoring and managing organoid viability is important for developing consistent and robust culture protocols. It is also essential when using 3D culture models for drug discovery or toxicology screening. Common techniques for evaluating cell viability include the MTT Assay, which is used to label metabolically active cells in intact and unfixed organoid tissue.

Single cell analysis can provide a more granular, and potentially more sensitive, assay of organoid viability when conducting drug or toxicology screening. The CometAssay™ and CometChip™ Assays enable high throughput single-cell detection of DNA damage in organoids. While methods for organoid tissue dissociation that minimizes cell damage are challenging and limitating for single cell techniques, R&D Systems has demonstrated that liver organoids show similar drug toxicity to hepatotoxic compounds using both MTT Cell Viability Assay as well as CometChip analysis.

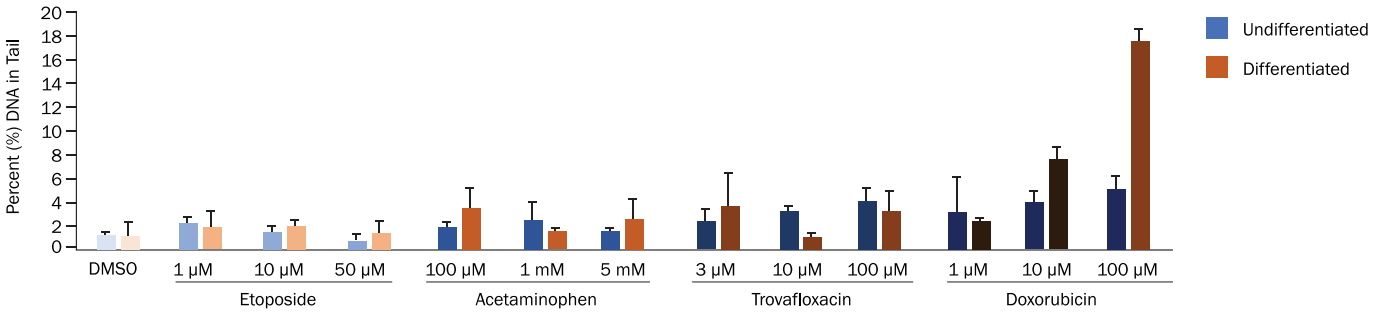


Figure 10. DNA CometChip Assay of Human Stem Tissue-derived Liver Organoids. Quantification of DNA damage showed toxicity only for cells treated with Doxorubicin (≥ 10 µM). Differentiated organoids were more sensitive to Doxorubicin treatment than undifferentiated organoids. No significant DNA damage was observed with the other hepatotoxic drugs.

Learn more | bio-techne.com/research-areas/organoids-3d-culture

Viability Assays | rndsystems.com/viability

Comet Assays | rndsystems.com/cometassay

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