# THE ORGANOID HANDBOOK

## BUILDING BETTER ORGANOIDS

bio-techne®

# 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, lung, 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.

# TABLE OF CONTENTS





# 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.

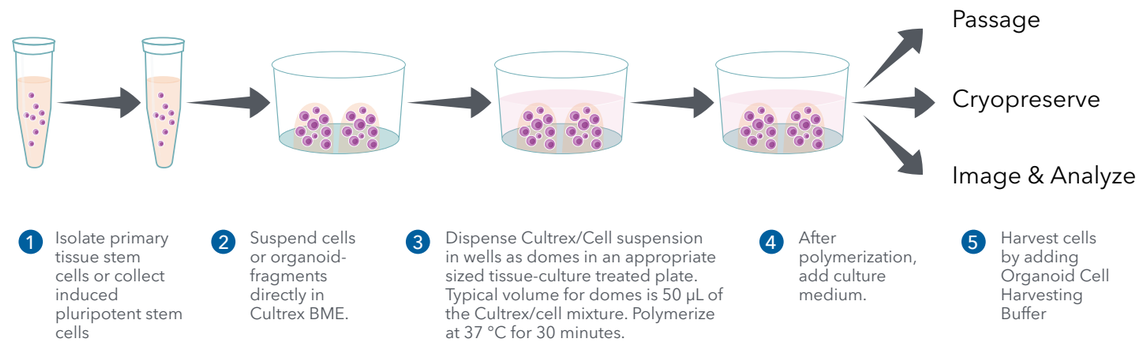



**FIGURE 1: General Schematic of Organoid Cell Culture.** Individual cells or organoid fragments are embedded within a liquid extracellular matrix (ECM) and dispensed as small droplets onto the surface of a warm tissue culture plastic vessel. The ECM will solidify into a gel after incubation at 37°C 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.

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 a robust and consistent organoid culture protocols.

Leading organoid researchers agree that reproducibility and culture longevity are the biggest challenges facing organoid biology. Some techniques recommended that can improve long-term culture quality and increase model consistency and 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.

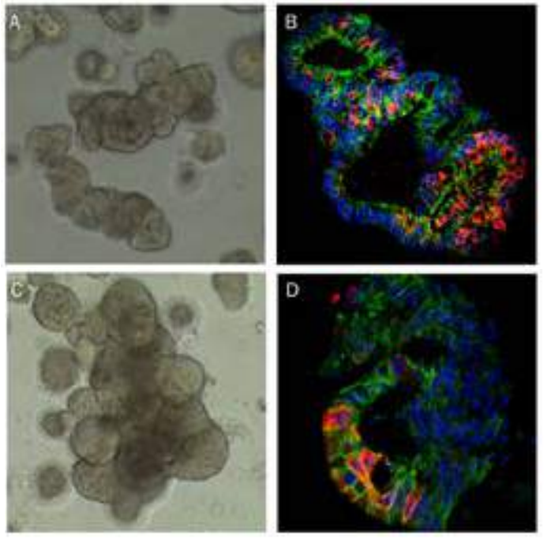
Learn more in our Virtual Organoid Symposium | bio-techne.com/webinars

*"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.

# 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 form iPSCs. iPSC-derived organoids have been used as advanced models for gastrointestinal developmental biology, drug toxicity, and personalized medicine applications.




**Human Intestinal Organoids Cultured using Cultrex RGF BME, Type 2.** Human transverse colon organoids **(A, B)** and human ileum organoids **(C, D)** were grown using cells isolated from transverse colon and ileum biopsy tissue, respectively. Organoids were embedded in Cultrex RGF BME, Type 2 (R&D Systems, Catalog # 3533-005-02) as a scaffold matrix. **A)** Brightfield image of human transverse organoids. **B)** Human transverse organoid stained using a Goat Anti-Human/Mouse E-Cadherin Antigen Affinity-purified Polyclonal Antibody (green; R&D Systems, Catalog # AF748), a Mouse Ant-Human MUC2 Monoclonal Antibody (red; Novus Biologicals, Catalog # NBP2-44431), and DAPI (blue; R&D Systems, Catalog # 5748). **C)** Brightfield image of human ileum organoids. **D)** Human ileum organoid stained using a Rabbit Anti-Human Aldolase B Polyclonal Antibody (red; Novus Biologicals, Catalog # NBP2-15345), a Mouse Anti-Human Cadherin-17 Monoclonal Antibody (green; R&D Systems, Catalog # MAB1032, and DAPI (blue; R&D Systems, Catalog # 5748).

## REAGENTS USED FOR INTESTINAL ORGANOID CULTURE

| PRODUCT NAME | CATALOG # |
|---|---|
| Cultrex Reduced Growth Factor Basement Membrane Extract (RGF BME), Type 2 | 3533-005-02 |
| GlutaminePlus | B90210 |
| HEPES | R35150 |
| N21-MAX Supplement | AR008 |
| N-2 MAX Supplement | AR009 |
| N-Acetylcysteine | 5619 |
| Gastrin I (Human) | 3006 |
| Y-27632 dihydrochloride (Rho Kinase Inhibitor) | 1254 |
| Recombinant Human EGF | 236-EG |
| Recombinant Human R-Spondin 1 | 4645-RS |
| Recombinant Human Noggin | 6057-NG |
| Recombinant Human FGF-2 | 3718-FB |
| A 83-01 | 2939 |
| CHIR 99021 | 4423 |
| Recombinant Human Wnt-3a | 5036-WN |

*"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 Puschof, Hans Clevers lab, Hubrecht Institute, The Netherlands.

## 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 BME, Type 2 | 3533-005-02 |
| | | A 83-01 | 2939 |
| | | CHIR 99021 | 4423 |
| Sato T. and H. Clevers (2015) Cell. **161**:1700 | Review of protocols for growing organoids from stem cells. | | |
| Jung P. et al. (2011) Nature Medicine. **17**:1225 | Isolation of human colonic stem cells. | | |



# 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 mechanism 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.

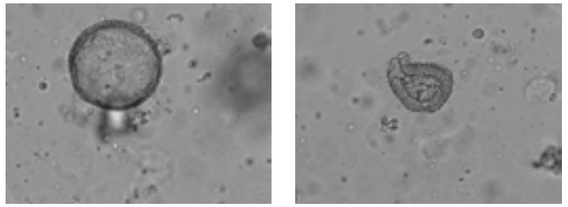


**FIGURE 1. Undifferentiated Human Gastric Organoids.** Representative brightfield images of human gastric organoids that were cultured using Cultrex RGF BME, Type 2 and the Bio-Techne reagents listed in this protocol.

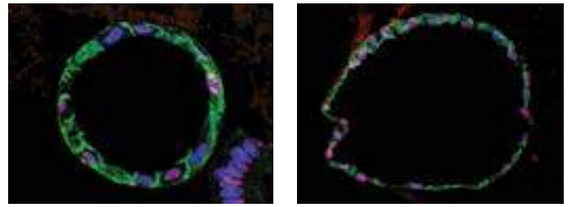


**FIGURE 2. Immunohistochemistry of Undifferentiated Human Gastric Organoids.** Human gastric organoids were cultured using Cultrex RGF BME, Type 2 and Bio-Techne reagents listed in this protocol. Undifferentiated colon organoids were stained using the Human/Mouse E-Cadherin Antibody (green; R&D Systems;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

## REAGENTS USED FOR GASTRIC ORGANOID CULTURE

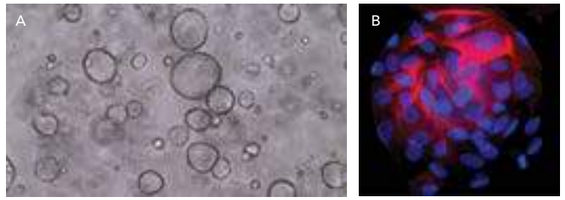
| REAGENT NAME | SUPPLIER | CATALOG # |
|---|---|---|
| Cultrex Organoid Harvesting Solution | R&D Systems | 3700-100-01 |
| Cultrex Reduced Growth Factor Basement Membrane Extract (RGF BME) Type 2 | R&D Systems | 3533-005-02 |
| Glutamine | Tocris Bioscience | 5823 |
| HEPES | Tocris Bioscience | 3173 |
| 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 |
| 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 |
| A 83-01 (ALK5 Inhibitor) | Tocris Bioscience | 2939 |
| CHIR 99021, (GSK-3 Inhibitor) | Tocris Bioscience | 4423 |
| Recombinant Human Wnt-3a | R&D Systems | 5036-WN |

## NOTABLE PUBLICATIONS AND PROTOCOLS

| PUBLICATION | DESCRIPTION | BIO-TECHNE REAGENTS USED | CATALOG # |
|---|---|---|---|
| McCracken, E.M. *et al*. (2014) Nature **516**:400.<br><br>McCracken, E.M. *et al*. (2017) Nature **541**:182. | Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. | Recombinant Human BMP4 | 314-BP |
| | | Recombinant Human FGF4 | 235-F4 |
| | | Recombinant Human Noggin | 6057-NG |
| | | Recombinant Human EGF | 236-EG |
| | | Recombinant Human FGF10 | 345-FG |
| | | Recombinant Human Wnt5a | 645-WN |
| Munero J. *et al*. (2017) Cell Stem Cell **21**:51. | iPSC differentiation into colonic organoids. | Y-27632 dihydrochloride | 1254 |
| | | Recombinant Human FGF4 | 235-F4 |
| | | Recombinant Human BMP2 | 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 adenocarcinoma. | Cultrex BME RGF Type 2 | 3533-005-02 |
| | | Cultrex HA-R-Spondin1-Fc 293T Cells | 3710-001-01 |
| | | A83-01 | 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.




**Human Liver Organoids. A)** Brightfield Image of human undifferentiated liver organoids cultured using Cultrex RGF BME, Type 2 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

| REAGENT NAME | SUPPLIER | CATALOG # |
|---|---|---|
| Cultrex Organoid Harvesting Solution | R&D Systems | 3700-100-01 |
| Cultrex Reduced Growth Factor Basement Membrane Extract (RGF BME), Type 2 | R&D Systems | 3533-005-02 |
| Glutamine | Tocris Bioscience | 5823 |
| 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 |
| 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 BMP7 | R&D Systems | 354-BP |
| Recombinant Human HGF | R&D Systems | 294-HG |
| Forskolin | Tocris Bioscience | 1099 |
| A 83-01 (ALK5 inhibitor) | Tocris Bioscience | 2939 |
| Recombinant Human Wnt-3a | R&D Systems | 5036-WN |
| DAPT | Tocris Bioscience | 2634 |
| Dexamethasone | Tocris Bioscience | 1126 |

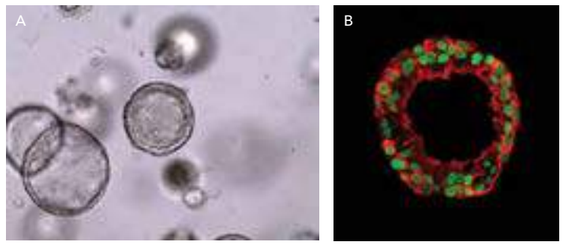
## NOTABLE PUBLICATIONS AND PROTOCOLS

| 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 BME, Type 2 | 3533-010-02 |
| | | A 83-01 | 2939 |
| | | Forskolin | 1099 |
| Ogawa, M. *et al.* (2015) Nat. Biotechnol. **33**:853. cc | Human iPSC-derived cholangiocyte organoids. | L-685,458 | 2627 |
| | | Recombinant Human HGF | 294-HG |
| | | Recombinant Human EGF | 236-EG |
| | | Recombinant Human TGF-ß1 | 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 BME, Type 2 | 3533-005-02 |
| Koike, H. *et al.* (2019) Nature 5**74**:112. | Modelling hepato-bili-ary-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.




**Human Lung Organoids. A)** Brightfield Image of human lung organoids cultured using Cultrex RGF BME, Type 2 and in media featuring Bio-Techne reagents. **B)** Expression of Sox2 (green; R&D Systems, Catalog # AF2018) and Acetylated Tubulin (red; Novus Biologicals, Catalog # NB600-567) in human lung organoids.

## REAGENTS USED FOR LUNG ORGANOID CULTURE

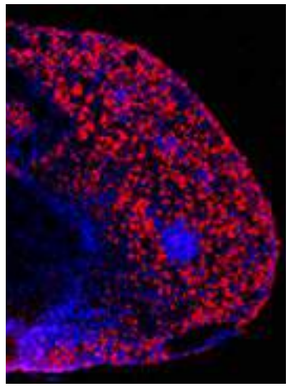
| REAGENT NAME | SUPPLIER | CATALOG # |
|---|---|---|
| A 83-01 | Tocris Bioscience | 2939 |
| Cultrex Organoid Harvesting Solution | R&D Systems | 3700-100-01 |
| Cultrex Reduced Growth Factor Basement Membrane Extract (RGF BME), Type 2 | R&D Systems | 3533-005-02 |
| Advanced DMEM/F-12 Cell Culture Medium | | |
| Glutamine | Tocris Bioscience | 5823 |
| HEPES | Tocris Bioscience | 3173 |
| N21-MAX Supplement | R&D Systems | AR008 |
| 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 |

## NOTABLE PUBLICATIONS AND PROTOCOLS

| PUBLICATION | DESCRIPTION | BIO-TECHNE REAGENTS USED | CATALOG # |
|---|---|---|---|
| Sachs, N. *et al.* (2019) EMBO J. **34**:e100300. | Protocol for adult stem cell-derived lung organoids. | Cultrex RGF BME, 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 FGF2 | 233-FB |
| | | Recombinant Human FGF4 | 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.



**Neural Progenitor Cells in Cerebral Organoids.** Human iPSCs were differentiated into cerebral organoids following the Lancaster protocol. Organoids were harvested and stained using a Human Pax6 Polyclonal Antibody (Catalog # AF8150) to identify neural progenitor cells in the developing cerebral tissue. Tissue was counterstained using DAPI (Catalog # 5748).

## REAGENTS USED FOR BRAIN ORGANOID CULTURE

| PRODUCT NAME | CATALOG # |
|---|---|
| BASE MEDIA COMPONENTS | |
| N-2 MAX Supplement | AR009 |
| N21-MAX Supplement | AR008 |
| N21-MAX Vitamin A Free Supplement | AR012 |
| Penicillin/Streptomycin | B21210 |
| GlutaMAX | B90210 |
| Insulin | |
| 2-mercaptoethanol | |
| MATRIX AND MEDIA ADDITIVES | |
| Cultrex RGF Basement Membrane Extract, Type 2 | 3533-005-02 |
| Recombinant Human FGF basic | 3718-FB |
| Recombinant Human Noggin | 6057-NG |
| Y-27632 dihydrochloride | 1254 |

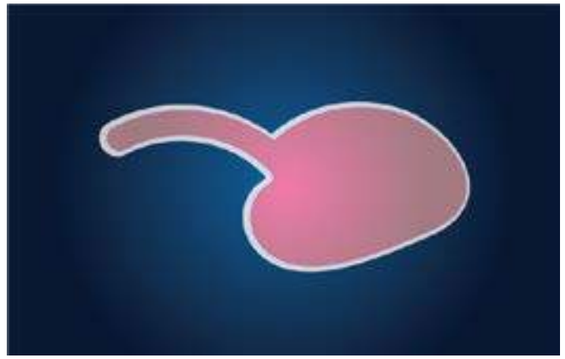
## NOTABLE PUBLICATIONS AND PROTOCOLS

| 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) Nature 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 | CATALOG # |
|---|---|
| BASE MEDIA COMPONENTS | |
| N-2 MAX Supplement | AR009 |
| N21-MAX Supplement | AR008 |
| N-Acetylcysteine | 5619 |
| Penicillin/Streptomycin | B21210 |
| GlutaMAX | B90010 |
| Holo-Transferrin | |
| Advanced DMEM/F-12 | |
| MATRIX AND MEDIA ADDITIVES | |
| Cultrex RGF Basement Membrane Extract, Type 2 | 3533-005-02 |
| Recombinant Human Activin A | 338-AC |
| Recombinant Human BMP-2 | 355-BM |
| Recombinant Human BMP-4 | 314-BP |
| Recombinant Human FGF basic | 3718-FB |
| Recombinant Human FGF-9 | 273-F9 |
| CHIR 99021 | 4423 |
| Retinoic Acid | 695 |
| Y-27632 . dihydrochloride | 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-9 | 273-F9 |
| | | Recombinant Human FGF-2 | 233-FB |
| 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 | 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

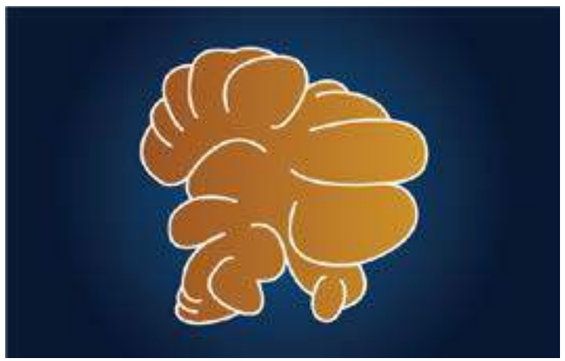
*In vitro* generation of cardiac tissue is enabling advancements in drug discovery and toxicity testing, as well as facilitating the engineering of cardiac tissue for regenerative therapies. Various methods have been employed to generate 3D cardiac tissue, including iPSC-derived cardiomyocyte spheroids 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 BME, Type 2 | 3533-005-02 |
| | | Recombinant Human R-Spondin 3 | 3500-RS |
| Rosenbluth, J.M. (2020) Nat. Comm. **1711**. | Human mammary organoids derived from breast tissue. | A83-01 | 2939 |
| Jamieson, P. *et al.* (2017) Stem Cells and Regeneration **144**:1065. | Mouse mammary organoids derived from epithelial cells. | Cultrex RGF BME, 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

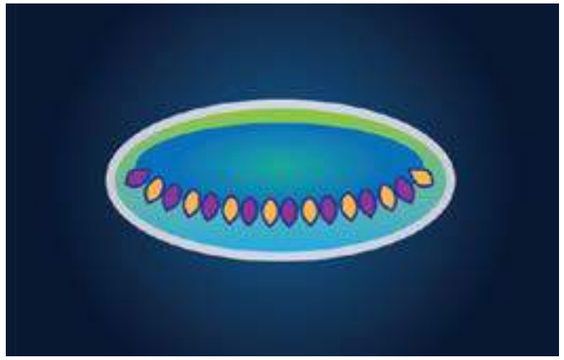
| PRODUCT NAME | CATALOG # |
|---|---|
| **BASE MEDIA COMPONENTS** | |
| N-2 MAX Supplement | AR009 |
| N21-MAX Supplement | AR008 |
| N-Acetylcysteine | 5619 |
| Penicillin/Streptomycin | B21210 |
| Glutamine | B90010 |
| Advanced DMEM/F-12 | |
| **MATRIX AND MEDIA ADDITIVES** | |
| Cultrex RGF Basement Membrane Extract, Type 2 | 3533-005-02 |
| Recombinant Human EGF | 236-EG |
| Recombinant Human FGF-10 | 345-FG |
| Recombinant Human Noggin | 6057-NG |
| Recombinant Human R-Spondin 1 | 4645-RS |
| Recombinant Human Wnt-3a | 5036-WN |
| A 83-01 | 2939 |
| Nicotinamide | 4106 |
| Gastrin | 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 BME, Type 2 | 3533-005-02 |
| | | PGE-2 | 2296 |
| | | Forskolin | 1099 |
| | | A83-01 | 2939 |
| Broutier, L. *et al.* (2016) Nat. Protocols **11**:1724. | Protocol to culture self-renewing human pancreatic organoids. | Cultrex RGF BME, Type 2 | 3533-005-02 |
| | | Cultrex HA-R-Spondin1-Fc 293T Cells | 3710-001-01 |
| | | Recombinant Human FGF-19 | 2939 |
| | | A83-01 | 969-FG |
| | | PGE-2 | 2296 |
| Greggio, C. *et al.* (2013) Development **140**:4452. | Effects of reagents and matrices on pancreatic organoid culture. | R-spondin 1 | 4645-RS |
| | | FGF-10 | 345-FG |
| | | FGF-2 | 233-FB |
| Dossena, M. *et al.* (2020) Stem Cell Research & Therapy **11**:94. | GMP-compliant culture of human pancreatic organoids. | R-spondin 1 | 4645-RS |
| | | A83-01 | 969-FG |
| | | 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.



## NOTABLE PUBLICATIONS AND PROTOCOLS

| PUBLICATION | DESCRIPTION |
|---|---|
| Koehler K.R. and E. Hashino (2014) Nat. Protocols. **9**:1229. | Inner ear organoids from mouse embryonic stem cells. |
| Liu, X. *et al*. (2016) Nat. Comm. **7**:11508. | Vestibular organoids from mouse embryonic stem cells. |
| Koehler, K.R. *et al*. (2017) Nat. Biotech. **35**:583. | Inner ear organoids from human induced pluripotent stem cells. |

## REAGENTS USED FOR INNER EAR ORGANOID CULTURE

| PRODUCT NAME | CATALOG # |
|---|---|
| LIF-2: BASE MEDIA COMPONENTS | |
| N-2 MAX Supplement | AR009 |
| N21-MAX Supplement | AR008 |
| Leukemia Inhibitor Factor (LIF) | 7734-LF |
| CHIR 99021 | 4423 |
| Penicillin/Streptomycin | B21210 |
| Glutamine | B90010 |
| Advanced DMEM/F-12 | |
| MATRIX AND MEDIA ADDITIVES | |
| Cultrex RGF Basement Membrane Extract, Type 2 | 3533-005-02 |
| Recombinant Human BMP-4 | 314-BP |
| Recombinant Human FGF basic | 3718-FB |
| A 83-01 | 2939 |



# 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.

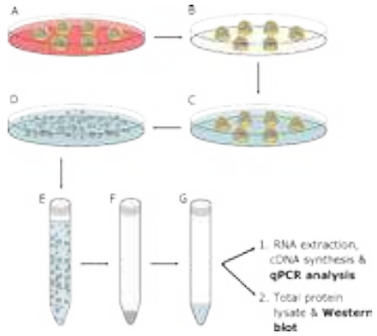



**Figure 1. Summarized Protocol to Harvest Organoids for Biochemical Analysis. A)** Treat organoids with differentiation medium. **B)** Discard medium. **C)** Add Cultrex Organoid Harvesting Solution. **D)** Incubate at 2–8 °C. **E)** Transfer organoids to a conical tube. **F)** Centrifuge organoids. **G)** Resuspend organoids in the appropriate lysis solution for either RNA extraction or protein analysis.

| PRODUCT NAME | CATALOG # |
|---|---|
| Cultrex Organoid Harvesting Solution | 3700-100-01 |

# 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.

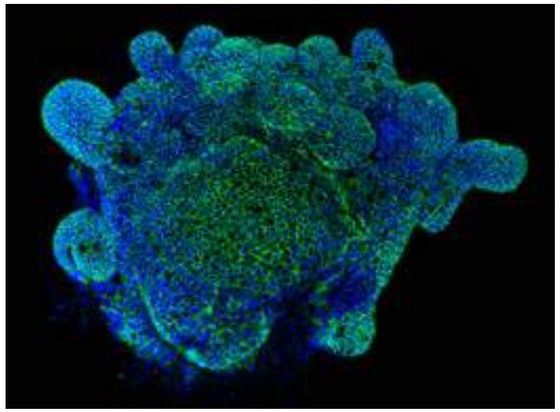
| PRODUCT NAME | CATALOG # | BRAND | DESCRIPTION |
|---|---|---|---|
| Fetal Bovine Serum – Premium Select | S11150 | R&D Systems | USDA-approved, Central American-orgin FBS |
| Fetal Bovine Serum – Optima | S12450 | R&D Systems | US-Origin, USDA-APHIS approved FBS |
| DMSO, sterile filtered | 3176 | Tocris | |

*Reference organoid cryopreservation protocol based on Taniguchi Laboratory at MD Anderson Cancer Center.

# 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 Clever 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 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.



**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/organoids

## 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.

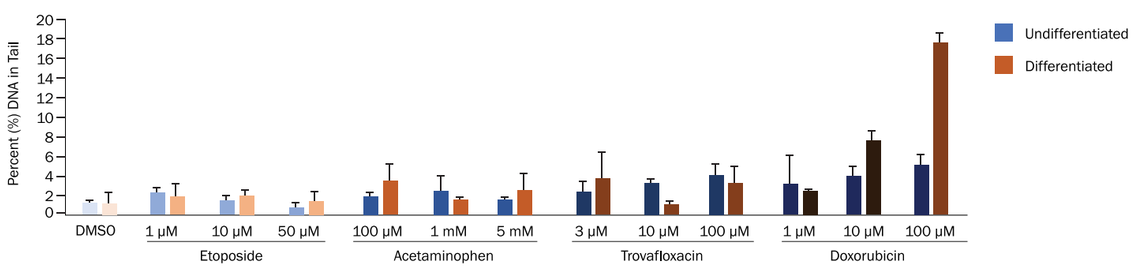
Learn more | acdbio.com

> *"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, US.

## 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.




**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.

Viability Assays | rndsystems.com/viability  Comet Assays | rndsystems.com/cometassay



NOTES