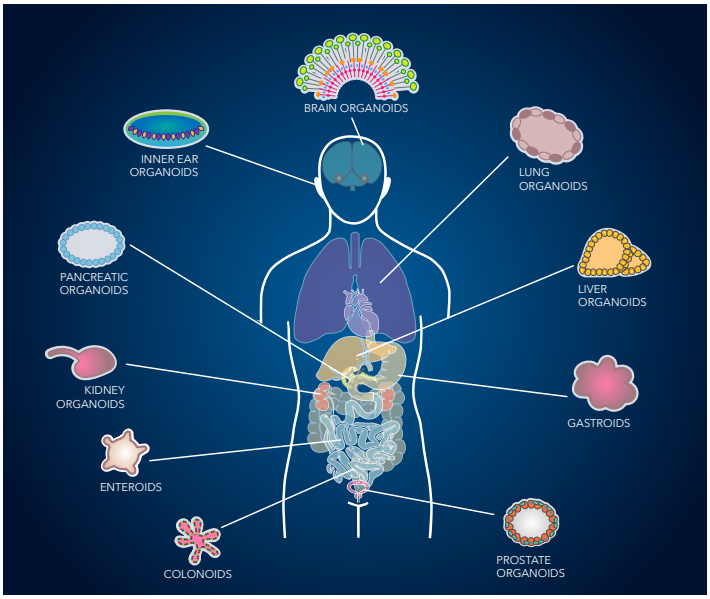
R&D SYSTEMS a bio-techne brand

APPLICATION NOTE

OPTIMIZING ORGANOID CULTURE CONDITIONS: THE IMPORTANCE OF GROWTH FACTOR BIOACTIVITY AND REAGENT CONSISTENCY

INTRODUCTION



An organoid is a miniature, simplified version of an organ produced *in vitro* that resembles the cellular composition and architecture of the tissue of origin and exhibits functional similarities. Organoids are derived from primary tissue, embryonic stem cells (ESCs), or induced pluripotent stem cells (iPSCs), which are capable of self-renewal and differentiation. Under specific culture conditions, these cells are driven to form 3-dimensional structures that self-organize into organ-like tissues. Organoids typically consist of multiple cell types that are correctly positioned with respect to both each other and the extracellular matrix and are present in a physiologically relevant microenvironment. They are also amenable to long-term expansion and manipulation. As a result, they are being increasingly used as *in vitro* model systems for studying human organ development, modeling disease conditions, screening for drug efficacy or toxicity, and investigating personalized medicine.

Organoid systems have been developed for a variety of different human tissues, including brain, colon, inner ear, intestine, kidney, liver, lung, pancreas, prostate, and retina, among others. Since the conditions for culturing different organoids are variable, the reagents and protocols needed for their generation have to be tested and optimized. Once optimal culture conditions are established, researchers must ensure that these conditions are reproducible so that the organoids can be successfully passaged, and the researcher can be confident that they are working on identical systems from one experiment to the next.

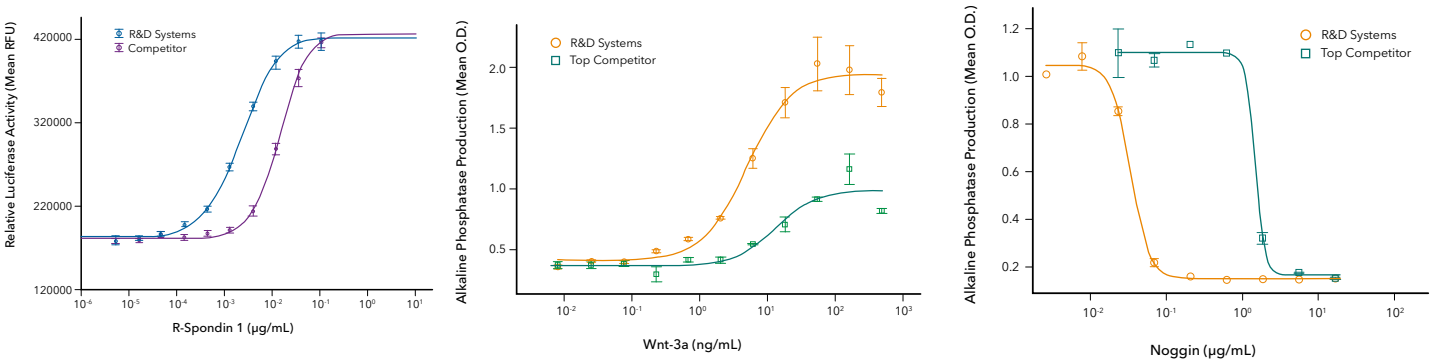
R&D SYSTEMS® IS THE MOST TRUSTED SOURCE FOR RECOMBINANT PROTEINS FOR ORGANOID RESEARCH

Some of the most important components of organoid media are growth factors such as R-Spondins, Noggin, and Wnt-3a, which need to display high levels of activity, batch-to-batch consistency, and be free of contaminants to ensure that they provide optimal, consistent organoid growth. Since these proteins can be difficult to make, it is critical that these reagents are produced or obtained from a reliable source to ensure that they maintain a high level of activity and consistency. This allows researchers to be confident that their experiments will be reproducible over time.

Many organoid researchers rely on R&D Systems as a trusted source for these reagents due to our rigorous in-house testing and quality control specifications. This is evidenced by the number of publications that cite the use of R&D Systems R-Spondins, Noggin, or Wnt-3a proteins for culturing organoids. Our recombinant human R-Spondin 1, Wnt-3a, and Noggin consistently show higher levels of bioactivity than competitors' proteins and each new lot is tested side-by-side with previous lots to ensure that the new lot displays the same level of bioactivity and purity as previous lots. New lots are also tested to make sure that they meet our expectations for endotoxin specifications, which is an industry-leading <0.1 EU/µg. With over 30 years of experience purifying and manufacturing recombinant proteins, we offer customers stability of source and the expertise necessary to ensure that our growth factors provide superior performance and minimal lot-to-lot variability.

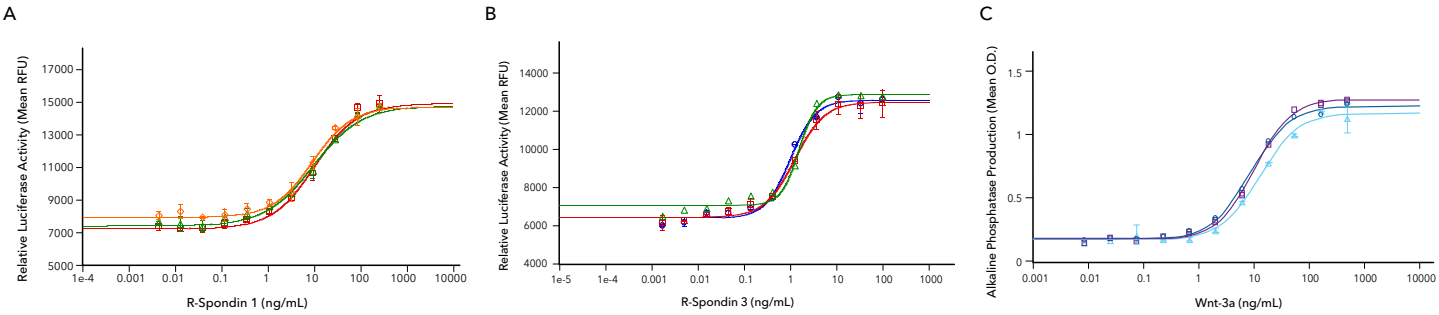
R&D SYSTEMS RECOMBINANT HUMAN R-SPONDIN 1, WNT-3A, AND NOGGIN DISPLAY HIGHER LEVELS OF ACTIVITY THAN LEADING COMPETITORS' PROTEINS

A B C

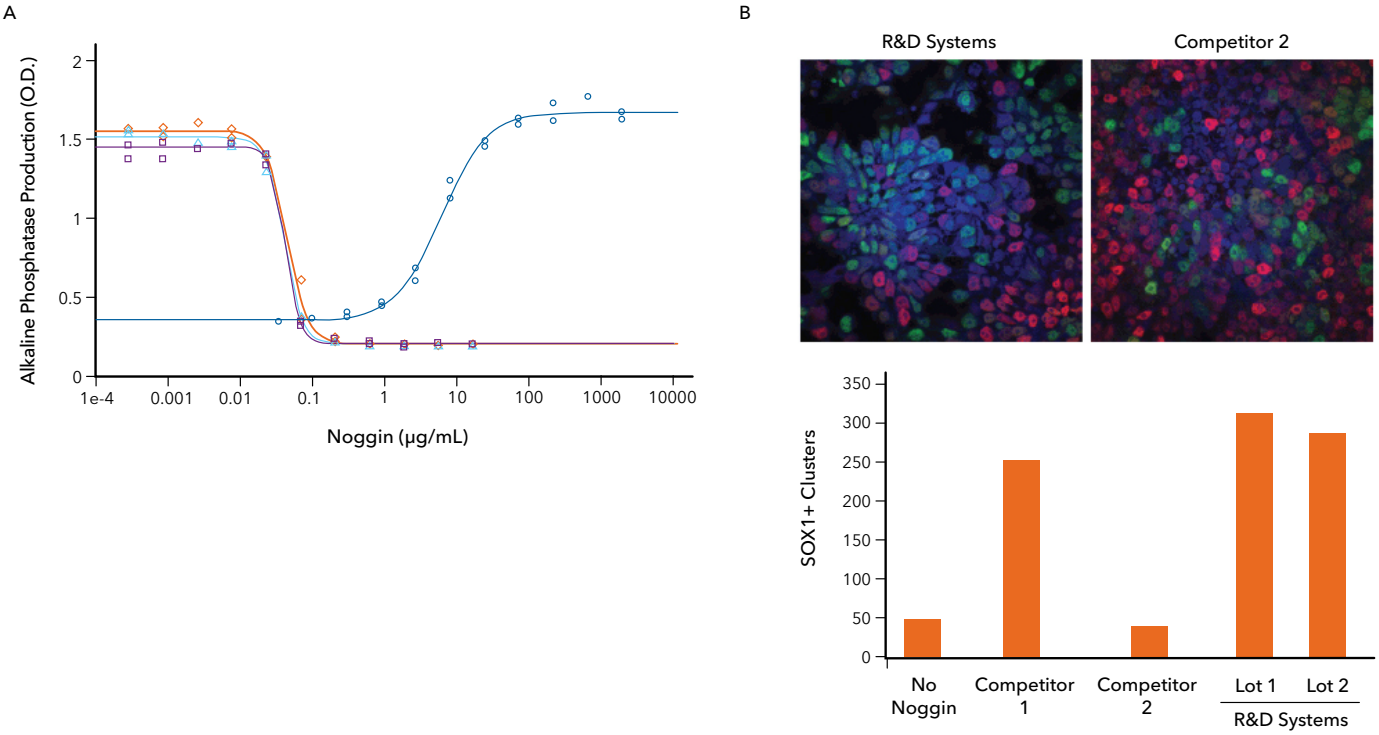


R&D Systems Recombinant Human R-Spondin 1, Wnt-3a, and Noggin Display Higher Activity than the Same Proteins Provided by Leading Competitors. (A) The ability of R&D Systems Recombinant Human R-Spondin 1 (Catalog # 4645-RS; blue line) or recombinant human R-Spondin 1 from a leading competitor (purple line) to stimulate activation of beta-Catenin using a TOPflash beta-Catenin/TCF reporter assay was tested in the HEK293T human kidney cell line, in the presence of Recombinant Mouse Wnt-3a (R&D Systems, Catalog # 1324-WN; 5 ng/mL). The ED_{50} for this effect for R&D Systems Recombinant Human R-Spondin 1 was approximately 7-fold greater than the competitor's R-Spondin 1. We also offer highly active Recombinant Human R-Spondin 3 (R&D Systems, Catalog # 3500-RS), which is being increasingly used for culturing different types of organoids. **(B)** The bioactivity of R&D Systems Recombinant Human Wnt-3a (Catalog # 5036-WN; orange line) or human Wnt-3a from another company (green line) was assessed by measuring the ability of the proteins to induce alkaline phosphatase production in the MC3T3-E1 mouse preosteoblast cell line. The ED_{50} for this effect of R&D Systems Recombinant Human Wnt-3a was 1.7-fold better, with more than twice the maximum response compared to the competitor's Wnt-3a. **(C)** The bioactivity of R&D Systems Recombinant Human Noggin (Catalog # 6057-NG; orange line) or recombinant human noggin from a top competitor (green line) was assessed by measuring the ability of the proteins to inhibit alkaline phosphatase production induced by 50 ng/mL Recombinant Human BMP-4 (Catalog # 314-BP) in the ATDC5 mouse chondrogenic cell line. The ED_{50} for this effect for R&D Systems Recombinant Human Noggin in the presence of 50 ng/mL of Recombinant Human BMP-4 was approximately 30-fold greater than the top competitor's Noggin.

R&D SYSTEMS RECOMBINANT HUMAN R-SPONDINS, WNT-3A, AND NOGGIN DISPLAY HIGH LOT-TO-LOT CONSISTENCY TO SUPPORT OPTIMIZED, CONSISTENT ORGANOID GROWTH



Lot-to-Lot Consistency Testing of Recombinant Human R-Spondin 1, R-Spondin 3, and Wnt-3a. Three independent lots of **(A)** Recombinant Human R-Spondin 1 (R&D Systems, Catalog # 4645-RS), or **(B)** Recombinant Human R-Spondin 3 (R&D Systems, Catalog # 3500-RS) were tested for their ability to stimulate activation of beta-Catenin using a TOPflash beta-Catenin/TCF reporter assay in the HEK293T human kidney cell line, in the presence of 5 ng/mL Recombinant Mouse Wnt-3a (R&D Systems, Catalog # 1324-WN). Each trace shown on the graph represents data obtained from Recombinant Human R-Spondin 1 or Recombinant Human R-Spondin 3 from a different manufacturing run. **(C)** Three independent lots of Recombinant Human Wnt-3a (R&D Systems, Catalog # 5036-WN) were tested for their ability to induce alkaline phosphatase production in the MC3T3-E1 mouse preosteoblast cell line. Each trace shown on the graph represents data obtained from Recombinant Human Wnt-3a from a different manufacturing run.



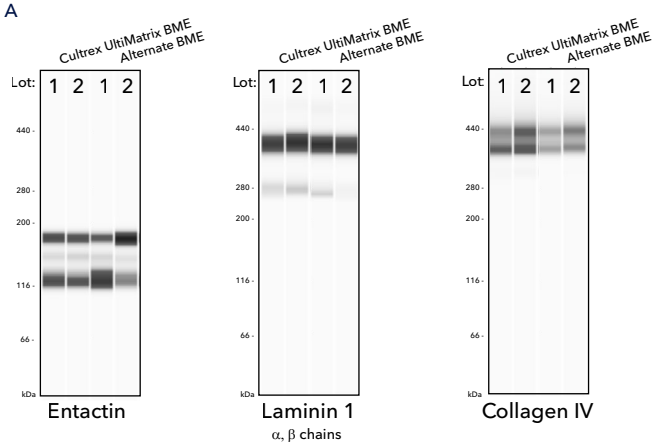
Lot-to-Lot Consistency Testing of Recombinant Human Noggin Demonstrates that R&D Systems Noggin Protein Displays Minimal Lot-to-Lot Variability and Higher Activity than Two Leading Competitors' Noggin Proteins. (A) Three independent lots of Recombinant Human Noggin (R&D Systems, Catalog # 6057-NG) were tested for their ability to inhibit alkaline phosphatase production induced by 50 ng/mL Recombinant Human BMP-4 (R&D Systems, Catalog # 314-BP) in the ATDC5 mouse chondrogenic cell line. Each trace shown on the graph (orange, light blue, purple) represents data obtained from Recombinant Human Noggin from a different manufacturing run. The blue trace shows the ability of Recombinant Human BMP-4 (50 ng/mL) to induce alkaline phosphatase production. **(B)** BG01V human embryonic stem cells were cultured in Mouse Embryonic Fibroblast Conditioned Media (R&D Systems, Catalog # AR005) supplemented with FGF basic (5 ng/mL). Stem cells were driven into early cells of the neuroectoderm using a 3-day incubation in Recombinant Human Noggin (25 µg/mL) from either R&D Systems (Lot 1, Lot 2; Catalog # 6057-NG) or from two separate competitors (Competitor 1, Competitor 2). Control cells were not incubated in Noggin (No Noggin). **(Top)** Representative images of embryonic stem cells that were stained for the early ectoderm marker, Otx2 (red), the neuroectoderm marker, SOX1 (green), and DAPI (blue), following differentiation with Noggin from R&D Systems or Noggin from Competitor 2. **(Bottom)** Quantification of SOX1+ clusters under each of the indicated culture conditions. Cells treated with R&D Systems Noggin showed an increase in SOX1+ cells compared to both untreated and competitor-treated cells. R&D Systems Noggin also showed consistent differentiation across the lots tested. BG01V human embryonic stem cells are licensed from ViaCyte, Inc.

BIO-TECHNE® OFFERS A COMPLETE PORTFOLIO OF REAGENTS DESIGNED TO PROMOTE THE ROBUST, REPRODUCIBLE EXPANSION OF ORGANOIDS

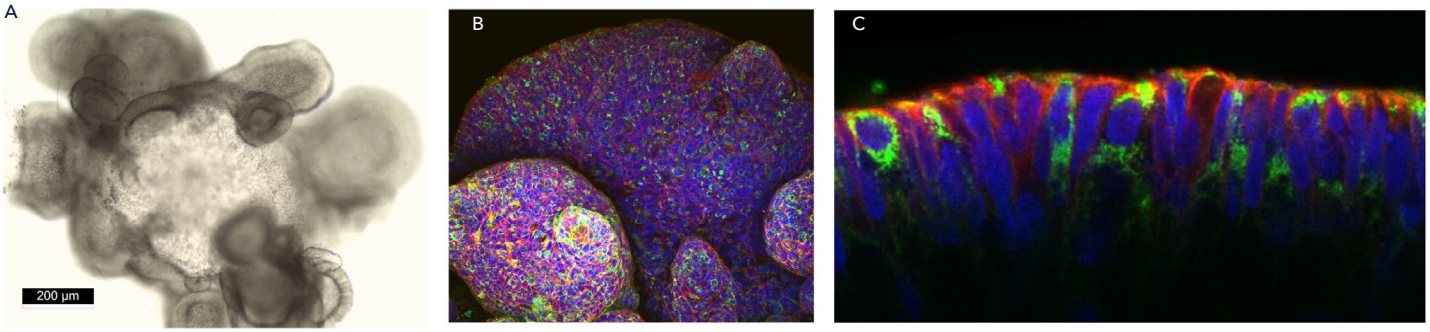
In addition to R&D Systems recombinant proteins, Bio-Techne offers a range of other products that are designed and tested to provide superior performance and maintain consistency during the culture, expansion, and passaging of organoids. These include different formats of organoid-qualified Cultrex™ Basement Membrane Extract (BME), Cultrex Organoid Harvesting Solution, media, extracellular matrix proteins, N-2 MAX and N-21 MAX media supplements, and small molecules commonly used in organoid cultures.

CULTREX™ ULTIMATRIX REDUCED GROWTH FACTOR (RGF) BASEMENT MEMBRANE EXTRACT

A number of different formats of Cultrex Basement Membrane Extract (BME) are available for 2-D and 3-D cell culture. Cultrex UltiMatrix Reduced Growth Factor (RGF) BME is a soluble form of basement membrane that provides high tensile strength, enhanced levels of entactin/nidogen, elevated protein concentration, and robust clarity and purity. These compositional enhancements translate into substantial performance benefits that make Cultrex UltiMatrix RGF BME an ideal extracellular matrix for consistent stem cell and organoid cell culture. View Our Complete Selection of Organoid-Qualified Basement Membrane Extracts | Cultrex BME & ECM Products



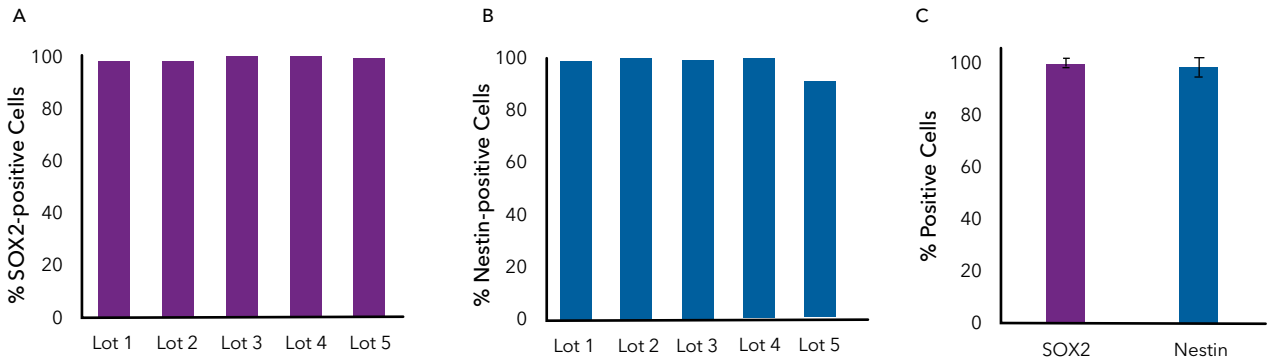
Cultrex UltiMatrix RGF Basement Membrane Extract (BME) Displays Consistent Lot-to-Lot ECM Composition, High Tensile Strength, and Gel Rates. A) Simple Western analysis for Entactin, Laminin, and Collagen IV across two lots of both Cultrex UltiMatrix RGF BME (R&D Systems, Catalog # BME001) and an alternate commercial BME matrix. Cultrex UltiMatrix RGF BME shows consistent expression of Entactin, compared to the alternate ECM matrix. **B)** The tensile strength (elastic modulus) dynamics across two lots of Cultrex UltiMatrix RGF BME and an alternate commercial BME matrix. The elastic modulus was measured at increasing temperatures to determine tensile strength and gelling rate across two independent lots of Cultrex UltiMatrix RGF BME (R&D Systems, Catalog # BME001) and the alternate BME matrix. Cultrex UltiMatrix RGF BME was found to display consistent gelling rates and high tensile strength (storage modulus) compared to the alternate commercial BME matrix.



Human Descending Colon Organoids Grown in Cultrex UltiMatrix RGF Basement Membrane Extract. 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 an Anti-Human Chromogranin A Monoclonal Antibody (green; R&D Systems, Catalog # MAB90981), to visualize intestinal enteroendocrine cells, and counterstained with an Anti-Human/Mouse E-Cadherin Antigen Affinity-purified Polyclonal Antibody (red; R&D Systems, Catalog # AF748) and DAPI (blue; R&D Systems, Catalog # 5748).

SERUM-FREE MEDIA SUPPLEMENTS

N-2 MAX and N21-MAX media supplements are also available to support organoid cultures. The N21-MAX Media Supplement improves upon the traditional B27- and NS21-like supplements, which are being increasingly used to optimize stem cell and 3-D cell culture media. Both the N21-MAX and N-2 MAX media supplements are serum-free, fully-defined supplements that are designed to eliminate uncontrolled variables, such as those found in serum-containing media and in some commercial serum-free supplements. These media supplements reduce unwanted experimental variations and have been shown to improve stem cell differentiation and increase the viability and health of differentiated cell types during long-term cell culture conditions.



Lot-to-Lot Consistency Testing of N-2 MAX Media Supplement. Rat cortical stem cells (RCSC) were cultured over multiple passages using N-2 MAX (R&D Systems, Catalog # AR009) and evaluated via flow cytometry for the maintenance of the stem cell markers, SOX2 and Nestin. Quantification of flow cytometry data demonstrates a high purity of **(A)** SOX2 or **(B)** Nestin positive RCSCs across multiple lots of N-2 MAX Supplement. **(C)** Average SOX2 and Nestin-positive RCSCs, demonstrate the consistent lot-to-lot performance of N-2 MAX.

SMALL MOLECULES FOR CULTURING ORGANOIDS

Bio-Techne also offers a wide range of Tocris® small molecules that are commonly used as 3-D growth matrix components for generating different types of organoids and sustaining long-term growth. These include products such as A 83-01, N-acetylcysteine, CHIR 99021, DAPT, dexamethasone, Gastrin I, nicotinamide, SB 202190, and more.

CULTREX ORGANOID HARVESTING SOLUTION

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

CRYOPRESERVATION MEDIA

Protein-free cryopreservation media for optimized cryopreservation of cell lines or cryopreservation of stem cells are also available. These specially formulated media contain a defined serum substitute as well as an optimized concentration of a cryopreservative that increases the recovery and viability of healthy cells compared to conventional freezing media.

ANTIBODIES AND RNASCOPE® *IN SITU* HYBRIDIZATION ASSAYS

Beyond our portfolio of reagents for organoid culture, we also offer a large selection of R&D Systems® antibodies and Novus Biologicals® antibodies for identifying lineage-specific markers, as well as RNAScope® *in situ* hybridization assays for detecting specific target RNAs in intact cells, when appropriate antibodies are not available.

> *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. Even for more exotic organoid, such as snake venom gland organoids, we could achieve breakthroughs with R&D Systems growth factors and BME as well as spatial visualization of toxin transcripts using ACD's RNAScope .*
>
> *Jens Puschof, Hans Clevers lab, Hubrecht Institute, The Netherlands.*

R&D SYSTEMS SCIENTISTS ARE DEVELOPING ORGANOID CULTURE PROTOCOLS AND OTHER ORGANOID-RELATED RESOURCES TO ASSIST RESEARCHERS

To further assist researchers that are working on generating organoids as model systems, scientists at R&D Systems are also working hard to optimize the culture conditions for growing different types of organoids. As we see success, we are publishing the lists of the reagents that we used, along with the recipes and protocols in our Organoid Resource Database, where they can be easily accessed by all researchers interested in utilizing organoids as model systems. As an example, the materials needed to culture human liver or lung organoids are shown below with links to our in-house protocols. In addition to these protocols, our organoid-related webinars, blogs, scientific posters, and literature are also available on our Organoid Resources page.

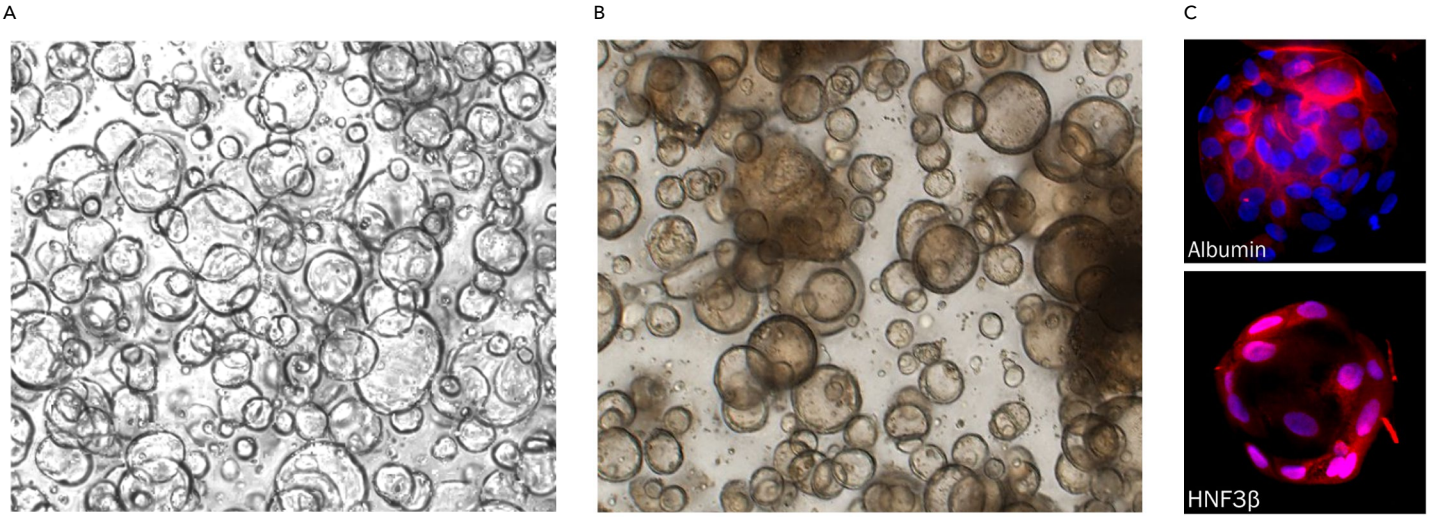
CULTURING HUMAN LIVER ORGANOIDS

TABLE 1. Materials needed for human liver organoid culture

REAGENT NAME	SUPPLIER	CATALOG #
Cultrex Organoid Harvesting Solution	R&D Systems	3700-100-01
Cultrex UltiMatrix Reduced Growth Factor Basement Membrane Extract	R&D Systems	BME001-05
Advanced DMEM/F-12 Cell Culture Medium	Thermo Fisher	12634-010
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

REAGENT 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 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

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



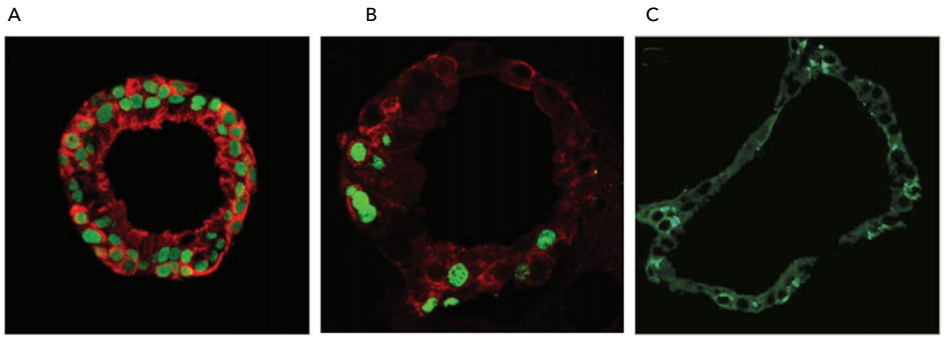
Undifferentiated and Differentiated Human Liver Organoids. Representative brightfield images of human **A**) undifferentiated and **B**) differentiated liver organoids that were cultured using Cultrex UltiMatrix RGF Basement Membrane Extract (R&D Systems, Catalog # BME001) and the Bio-Techne reagents listed in the Materials table shown above for culturing human liver organoids. The organoids were differentiated in media containing Recombinant Human FGF-19 (R&D Systems, Catalog # 969-FG), DAPT (Tocris, Catalog # 2634), and Dexamethasone (Tocris, Catalog # 1126). **C**) Undifferentiated liver organoids shrink as they differentiate and have positive expression of the hepatocyte markers, Albumin (**C**, top) and HNF-3beta (**C**, bottom), which were detected using a Mouse Anti-Human Serum Albumin Monoclonal Antibody (top, red; R&D Systems, Catalog # MAB1455) and a Goat Anti-Human HNF-3beta Antigen Affinity-purified Polyclonal Antibody (bottom, red; R&D Systems, Catalog # AF2400), respectively. DAPI (R&D Systems, Catalog # 5748) was used as a counterstain in part C of the figure (blue).

CULTURING HUMAN LUNG ORGANOIDS

TABLE 1. Materials needed for human lung organoid culture

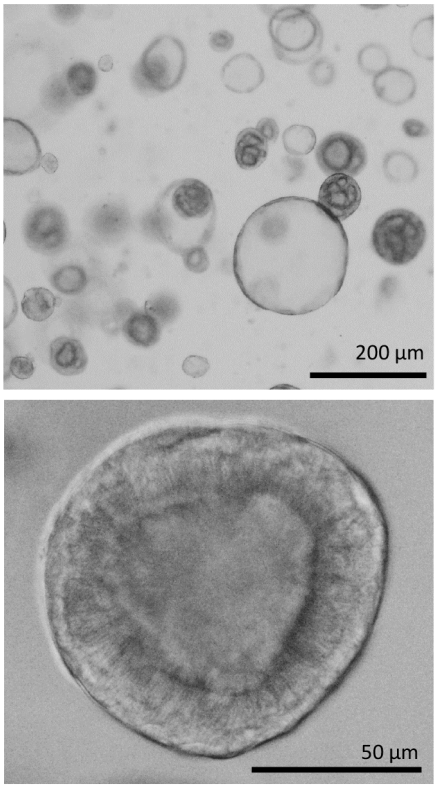
REAGENT NAME	SUPPLIER	CATALOG #
A 83-01	Tocris Bioscience	2939
Cultrex Organoid Harvesting Solution	R&D Systems	3700-100-01
Cultrex UltiMatrix Reduced Growth Factor Basement Membrane Extract	R&D Systems	BME001-05
Advanced DMEM/F-12 Cell Culture Medium	Thermo Fisher	12634-010
Glutamine	Tocris Bioscience	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

The complete Human Lung Organoid Culture protocol available in our Organoid Resources Database.



Characterization of Human Lung Organoids. Representative images of human lung organoids for tissue-specific cell types. **A**) Expression of SOX2 (green) and Acetylated Tubulin (red) was assessed using an Anti-Human/Mouse/Rat SOX2 Antigen Affinity-purified Polyclonal Antibody (R&D Systems, Catalog # AF2018) and an Anti-Acetylated Tubulin Monoclonal Antibody (Novus Biologicals, Catalog # NB600-567), respectively. **B**) Expression of p63/TP73L (green) and Cytokeratin 10 (green) was assessed using an Anti-Human p63/TP73L Antigen Affinity-purified Polyclonal Antibody (R&D Systems, Catalog # AF1916) and an Anti-Cytokeratin 10 Monoclonal Antibody (Novus Biologicals, Catalog # NBP2-61736), respectively. **C**) Expression of Podoplanin (green), a marker of type 1 alveolar cells, was assessed using an Anti-Human Podoplanin Antigen Affinity-purified Polyclonal Antibody (R&D Systems, Catalog # AF3670).

To view our complete collection of Organoid-related webinars, blogs, scientific posters, and literature, please visit our Organoid Resources Database webpage.



Human Lung Organoids Grown in Cultrex UltiMatrix RGF Basement Membrane Extract (BME). Representative images of lung organoids, derived from lung biopsy adult stem cells, embedded in Cultrex UltiMatrix RGF BME (R&D Systems, Catalog # BME001) and cultured in Lung Organoid Expansion Media. Images show organoids at day 52 of culture.

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