

ORGANOIDS

Robust Media to Culture
Intestinal, Hepatic,
Pancreatic, Kidney, Airway,
and Cerebral Organoids

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Introduction

One of the most impactful advances in stem cell research of the past decade has been the development of organoid culture techniques. Organoids are three-dimensional cell cultures that recapitulate some of the key cell types and structural features of the represented organ. These “mini-organs” enable scientists to investigate complex research questions *in vitro* by providing highly relevant tissue model systems. Since landmark publications describing intestinal¹ and cerebral organoids,² organoid culture techniques have been developed for multiple species and tissues to derive organoids from either adult stem cells (ASCs) or pluripotent stem cells (PSCs).

While each culture system is distinct, organoids generally contain multiple different organ-specific cell types capable of recapitulating some functions of that organ. During organoid culture, stem cells (PSCs or ASCs) are provided with appropriate signaling factors that mimic the *in vivo* environment. Exposure to these conditions directs stem cells to divide and differentiate into the cell types of the represented organ. In general, organoids display architecture similar to what is observed *in vivo*, such as polarization of the apical and basolateral surfaces (epithelial organoids), appropriate cellular stratification (cerebral organoids), or re-creation of complex domains such as the intestinal crypt-villus structure observed in mouse intestinal organoids. In epithelial organoid systems, the presence of actively dividing adult stem cells enable the cultures to be expanded and maintained in long-term culture through serial passaging.

Organoid cultures have seen quick and widespread adoption for a variety of applications, from basic research to translational and industrial uses. The roots of organoid technology lie in developmental biology, and organoids are now feeding back valuable information about the development of the tissues that they model, especially in the case of PSC-derived organoids. As organoids recapitulate many of the signaling and cell-cell interactions observed *in vivo*, they are also invaluable models for studying cell biology, including investigation into tissue regeneration mechanisms, maintenance of the stem cell niche, and interactions with bacteria, viruses, and cells from other tissues.

Removal of confounding variables inherent in animal models while providing increased complexity compared with homogeneous cell cultures gives organoid cultures specific experimental advantages. By combining a high level of physiological relevance with amenability to manipulation *in vitro*, organoids have the potential to complement or replace *in vitro* experiments using primary cells or immortalized cell lines. There is also a movement to use organoids to replace animal experimentation in many circumstances. Additionally, organoids demonstrate high genetic stability in culture, retaining the genotype and phenotype of the source tissue. Organoids therefore provide faithful models of disease and have been used to model disease mechanisms and progression, as well as to predict patient-specific response to drug treatment.

Continued development of organoid methodologies is proving transformative to the stem cell research landscape. In many labs, organoids have complemented model systems already in place, while in others they have enabled investigations previously not possible. Development and standardization of organoid culture techniques will enable even greater accessibility of organoids.

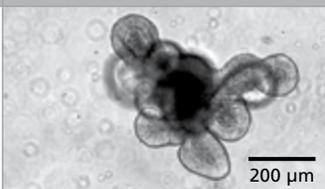
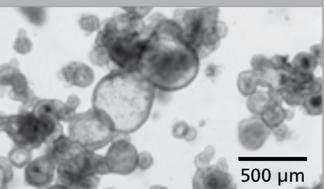
Intestinal Organoids

Intestinal organoids incorporate key intestinal cell types including enterocytes, goblet cells, enteroendocrine cells, transit amplifying cells, and intestinal stem cells, recapitulating the cellular complement of the intestinal epithelium. The presence of an actively dividing stem cell population enables expansion and maintenance of intestinal organoids in long-term culture.

IntestiCult™ Organoid Growth Medium (Mouse) and IntestiCult™ Organoid Growth Medium (Human) support establishment and

long-term maintenance of ASC-derived intestinal organoids from mouse and human cells, respectively. STEMdiff™ Intestinal Organoid Kit can be used to direct human PSCs through a three-stage differentiation process to intestinal organoids that can be maintained and matured in STEMdiff™ Intestinal Organoid Growth Medium. Intestinal organoids grown using these media are convenient, flexible, and relevant intestinal tissue models that can increase experimental impact and reduce laboratory animal use.

Table 1. Comparison of Intestinal Organoid Culture Systems

INTESTINAL ORGANOID TYPE	MOUSE (ASC-DERIVED)	HUMAN (ASC-DERIVED)	HUMAN (PSC-DERIVED)
Representative Image			
Organoid Cellular Composition	Organoids simultaneously incorporate an active intestinal stem cell compartment and differentiated cell types in an epithelium that recapitulates the in vivo intestine.	After 1 - 3 passages organoids are composed primarily of cells in a progenitor/stem-like state; organoids can be differentiated to mature intestinal cells in an appropriate medium.	Organoids simultaneously incorporate an active intestinal stem cell compartment, differentiated intestinal epithelial cell types and associated mesenchymal cells. Organoids are phenotypically fetal.
Starting Material	<ul style="list-style-type: none"> • Mouse intestinal or colonic crypts • Mouse Intestinal Organoids (Catalog #70931) 	<ul style="list-style-type: none"> • Human intestinal or colonic crypts • Established ASC-derived intestinal organoids 	<ul style="list-style-type: none"> • Human induced pluripotent stem cells (iPSC) or embryonic stem cell (ESC) lines • Established PSC-derived intestinal organoids
Organoid Maintenance	Organoids can be maintained through long-term passaging or cryopreserved.	Organoids can be maintained through long-term passaging or cryopreserved.	Organoids can be maintained through long-term passaging or cryopreserved.
Genetic Considerations	<ul style="list-style-type: none"> • Established common healthy and disease model strains • Tools for in vivo genetic manipulation 	<ul style="list-style-type: none"> • Donor-specific genetic background • Targeted in vitro gene editing possible 	<ul style="list-style-type: none"> • Donor-specific genetic background • Targeted in vitro gene editing possible
Compatible Culture Media	IntestiCult™ Organoid Growth Medium (Mouse) (Catalog #06005)	IntestiCult™ Organoid Growth Medium (Human) (Catalog #06010) IntestiCult™ Organoid Differentiation Medium (Human) (Catalog #100-0214); for differentiation in 3D organoids and as monolayers in submerged or air-liquid interface cultures.	STEMdiff™ Intestinal Organoid Kit (Catalog #05140) STEMdiff™ Intestinal Organoid Growth Medium (Catalog #05145)

IntestiCult™ Organoid Growth Medium (Mouse)

Cell Culture Medium for Establishment and Maintenance of Mouse Intestinal Organoids

IntestiCult™ Organoid Growth Medium (Mouse) is a complete, serum-free organoid growth medium that enables researchers to easily and reproducibly generate experiment-ready organoids in 5 - 7 days.

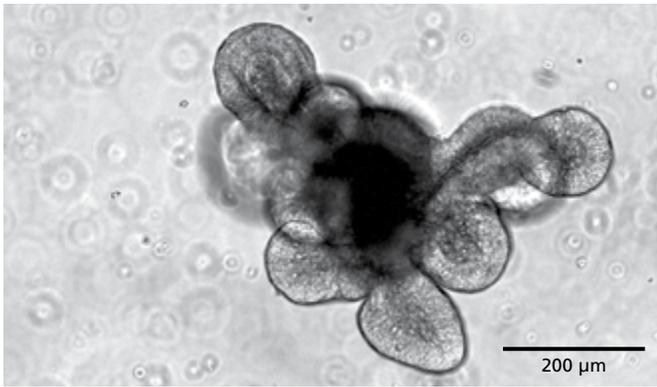


Figure 1. Light Microscope Visualization of a Mouse Intestinal Organoid

Mouse intestinal organoids were cultured in IntestiCult™ Organoid Growth Medium (Mouse) and imaged on day 5 of passage 0.

Why Use IntestiCult™ Organoid Growth Medium (Mouse)?

RELEVANT. Enables generation of intestinal organoid cultures that recapitulate the identity and organization of the adult intestinal epithelium.

ROBUST. Supports efficient establishment of organoids from mouse intestinal crypts in less than one week.

SIMPLE. Convenient format and easy-to-follow protocol.

SERUM-FREE. Optimized formulation for low experimental variability.

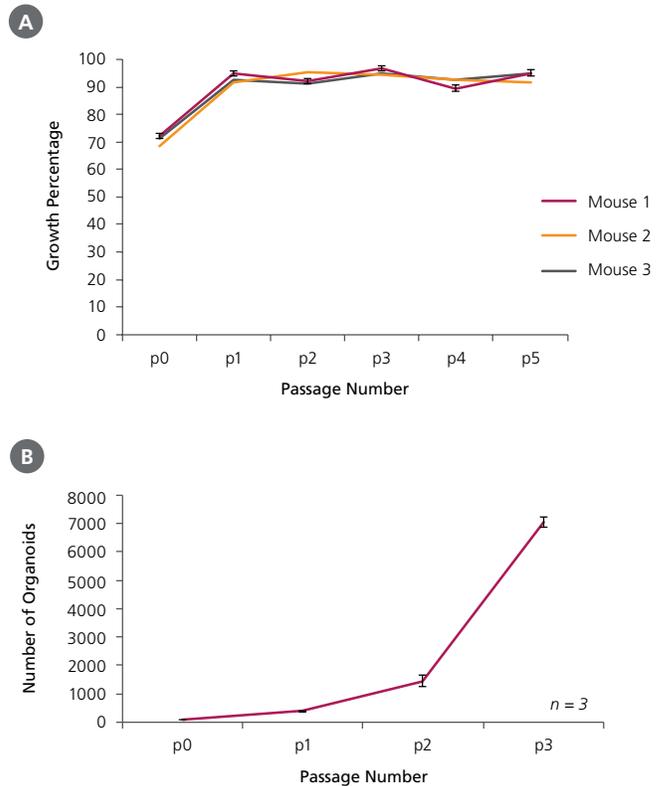


Figure 2. IntestiCult™ Organoid Growth Medium (Mouse) Supports Efficient Organoid Establishment and Expansion

(A) Established organoids can be passaged efficiently over an indefinite number of passages. (B) Starting from a single well containing 100 organoids and passaging at a 1 in 4 split ratio, organoid count increases an average of 4.2-fold per passage.

IntestiCult™ Organoid Growth Medium (Human)

Cell Culture Medium for Establishment and Maintenance of Human Intestinal Organoids

IntestiCult™ Organoid Growth Medium (Human) is a complete cell culture medium for efficient establishment and long-term maintenance of human intestinal epithelial organoids derived from primary intestinal or colonic crypts or previously frozen colonic organoids.

Why Use IntestiCult™ Organoid Growth Medium (Human)?

ROBUST. Reliable and efficient expansion of intestinal stem cells across donor samples.

RELEVANT. Enables generation of intestinal organoid cultures that can be used to model the adult intestinal epithelium.

COMPLETE. Two-component medium system does not require additional growth factors to support organoid growth.

CONSISTENT. Quality-controlled medium generates consistent results between experiments and between laboratories.

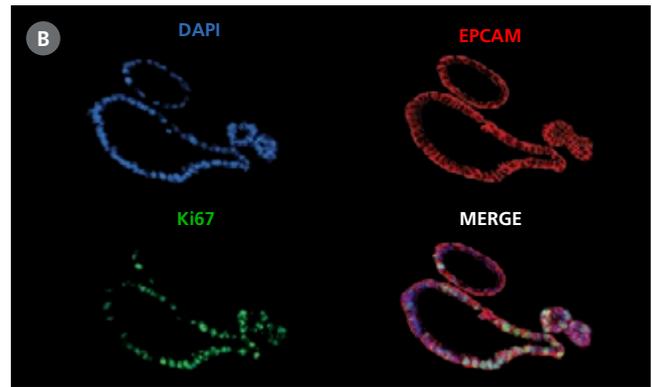
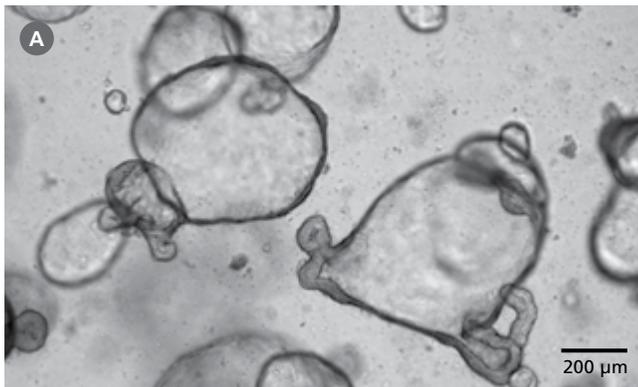


Figure 3. Human Colonic Organoids Grown in IntestiCult™ Organoid Growth Medium (Human)

(A) Colonic organoids grown in IntestiCult™ Organoid Growth Medium (Human) and imaged on day 7 of passage 0. (B) Immunofluorescence of intestinal organoids showing DAPI (blue), EPCAM (red), Ki67 (green), and the merged image.

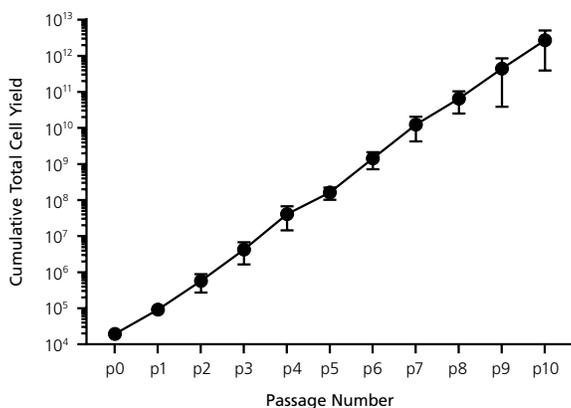


Figure 4. Intestinal Organoids Can be Maintained in Long-Term Culture Through Passaging

Organoids cultured in IntestiCult™ Organoid Growth Medium (Human) show efficient growth throughout passaging. Cultures were split with an average split ratio of 1 in 6 at each passage. Error bars represent standard error.

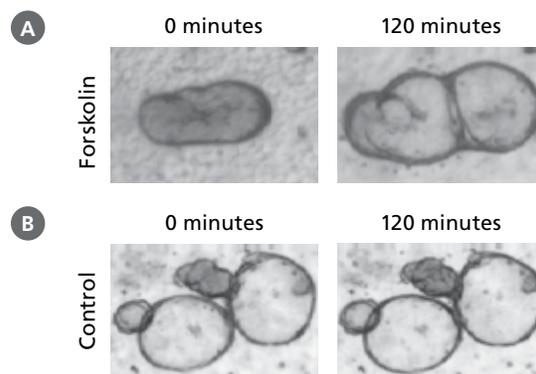


Figure 5. Forskolin-Induced Swelling of Intestinal Organoids

Organoids cultured in IntestiCult™ Organoid Growth Medium (Human) were treated with (A) 5 μM Forskolin or (B) DMSO. Organoid area was measured at 0 minutes and 120 minutes. Forskolin-treated organoids increased in size by 33.5 ± 3.8% in size compared to a 7.5 ± 0.8% increase for control organoids (n=3). Representative images are shown.

IntestiCult™ Organoid Differentiation Medium (Human)

Complete Culture Medium for the Differentiation of Intestinal Organoids.

IntestiCult™ Organoid Differentiation Medium (Human; ODM) is a complete culture medium that supports the further differentiation of intestinal organoids in three dimensions (3D), or in 2D as submerged monolayers or air-liquid interface (ALI) cultures. These cultures can be initiated from intestinal organoids derived from human intestinal crypts, or passaged organoids that have been cultured with IntestiCult™ Organoid Growth Medium (Human; OGM; Catalog #06010).

Why Use IntestiCult™ Organoid Differentiation Medium?

RELEVANT. Enables generation of intestinal organoid cultures with physiological proportions of differentiated and stem cell populations.

FLEXIBLE. Allows easy access to the apical surface by transitioning intestinal organoids into monolayer and ALI cultures.

REPRODUCIBLE. Generates consistent results across passages and replicates.

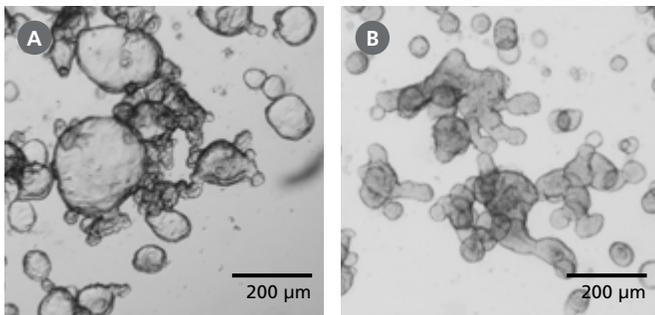


Figure 6. Differentiated Human Intestinal Organoids Display a Budded Morphology

(A) Organoids grown in IntestiCult™ OGM are primarily cystic. (B) When switched to IntestiCult™ ODM, organoids develop a thickened epithelium with a pronounced, budded morphology indicative of a more differentiated state. Organoids were imaged on day 5 of expansion or differentiation respectively.

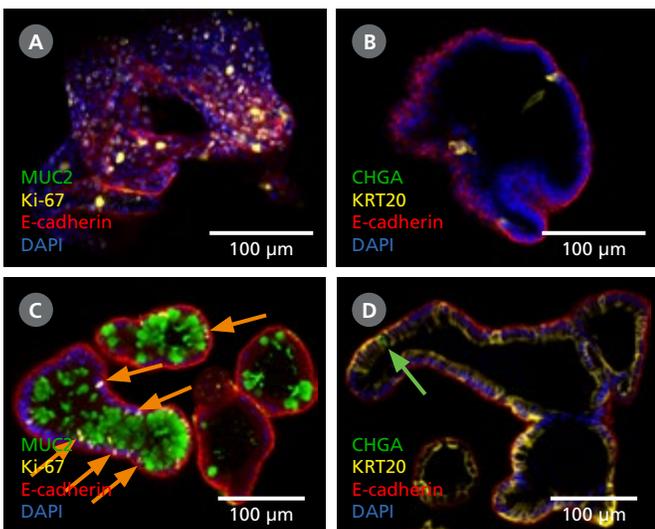


Figure 7. Intestinal Organoids Contain a Higher Proportion of Mature Cell Types Following Differentiation in IntestiCult™ ODM

(A, B) Organoids grown in IntestiCult™ OGM are enriched for Ki-67⁺ proliferative cells (A), while containing few differentiated cell types such as goblet cells (MUC2, A), enterocytes (KRT20, B), and enteroendocrine cells (CHGA, B). (C, D) When switched to IntestiCult™ ODM, organoids contain a small number of Ki-67⁺ proliferative cells (C, arrows), with more physiological proportions of goblet cells (MUC2, C), enterocytes (KRT20, D), and chromogranin A- (CHGA-)positive enteroendocrine cells (D, arrow).

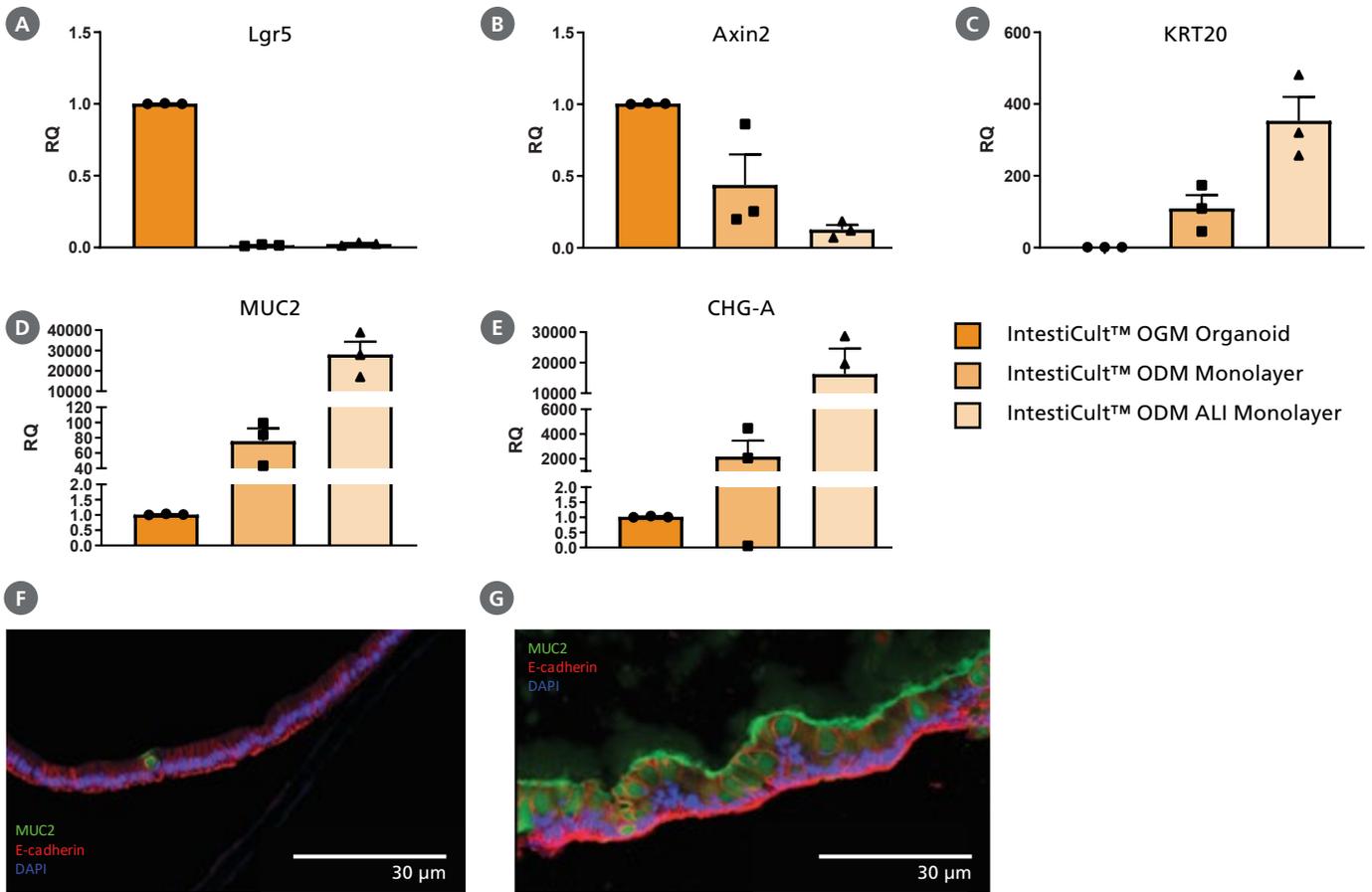


Figure 8. Differentiation of Intestinal Epithelium at the Air-Liquid Interface (ALI) Using IntestiCult™ ODM

(A – E) Growing organoid-derived monolayers as ALI cultures drives further differentiation of intestinal epithelial cultures as seen by changes in gene expression measured by RT-qPCR. Relative quantification (RQ) for each marker is shown relative to actB and TBP housekeeping genes and normalized with respect to undifferentiated organoids grown in IntestiCult OGM (Human). Progenitor markers (A) Lgr5 and (B) Axin2 are significantly reduced in both submerged monolayers and ALI cultures, while markers of enterocytes (KRT20, C), goblet cells (MUC2, D), and enteroendocrine cells (CHGA, E) are significantly increased. Further reduction in Axin2 is seen in ALI monolayers with an increase in expression of KRT20, MUC2, and CHGA. (F, G) Comparing cross-sections of organoid monolayers grown in IntestiCult™ ODM as (F) submerged culture or (G) at the ALI shows further differentiation of the intestinal epithelium with an increased proportion of goblet cells and extracellular mucus (MUC2, green).

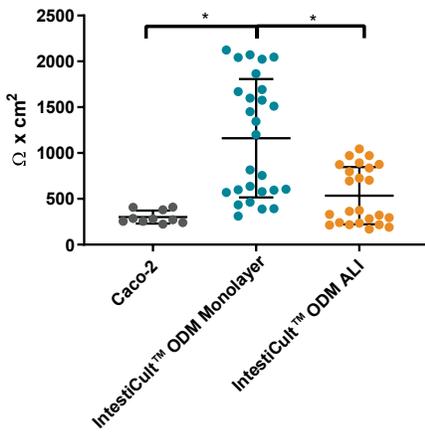


Figure 9. Differentiated Organoid-Derived Monolayers and ALI Cultures Display More Physiological Trans-Epithelial Electrical Resistance (TEER) than Caco-2 Cells

Differentiated organoid-derived monolayers grown as a submerged monolayer (IntestiCult™ ODM Monolayer), or at the ALI (IntestiCult™ ODM ALI), show higher TEER values as compared to Caco-2 cultures. Organoid-derived monolayers grown at the ALI show a loosening of tight junctions due to further differentiation of the brush border, and thus lower TEER values are observed. * $p < 0.0001$

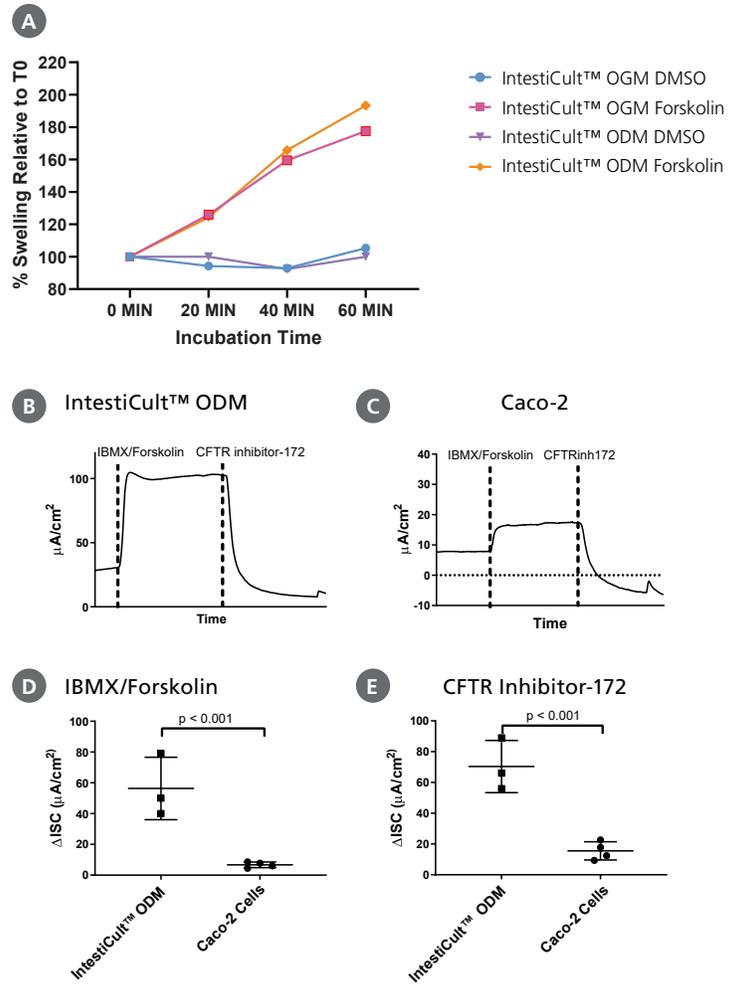


Figure 10. Differentiated Intestinal Organoids Provide a Suitable Model for Studying CFTR Response In Vitro

(A) Organoids differentiated further in IntestiCult™ ODM show a comparable degree of swelling when treated with forskolin as compared to organoids grown in IntestiCult™ OGM, demonstrating suitability for use in forskolin-induced swelling assays. (B – E) Ussing chamber analysis of submerged (B) organoid-derived monolayers and (C) Caco-2 cultures demonstrate increased sensitivity of organoid-derived monolayers to CFTR activation and inhibition by IBMX/Forskolin and CFTR Inhibitor-172 respectively. (D, E) Analysis of CFTR modulation by IBMX/Forskolin and CFTR Inhibitor-172 show significantly greater (D) activation and (E) inhibition of CFTR activity in organoid-derived monolayers as compared to Caco-2 cultures ($p < 0.001$ for both).

STEMdiff™ Intestinal Organoid Kit

Cell Culture Medium Kit for Differentiation of PSC-Derived Human Intestinal Organoids

The STEMdiff™ Intestinal Organoid Kit supports efficient establishment of PSC-derived small intestinal organoid cultures from embryonic stem (ES) cells or induced pluripotent stem (iPS) cells within 30 days. This serum-free medium kit is based on the formulation published by Spence et al.³ and has been optimized to increase efficiency and reproducibility of organoid formation and expansion.

Why Use STEMdiff™ Intestinal Organoid Kit?

RELEVANT. Enables generation of small intestinal organoid cultures that model the developing intestinal epithelium and associated mesenchyme.

ROBUST. Supports efficient differentiation of human ES and iPS cell lines to intestinal organoids.

CONVENIENT. Intestinal organoids can be maintained long-term through passaging or cryopreserved for experimental flexibility.

SERUM-FREE. Optimized formulation for low experimental variability.

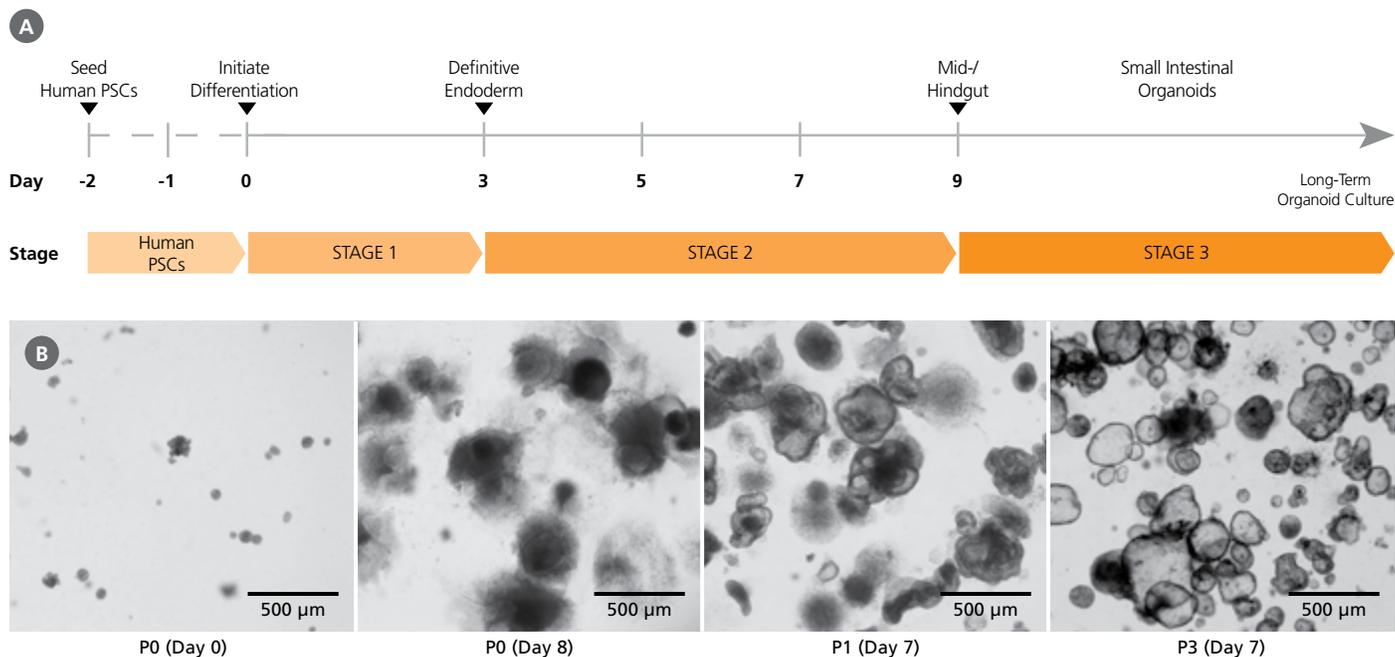


Figure 11. Generation of Human Intestinal Organoid Cultures Using the STEMdiff™ Intestinal Organoid Kit

(A) Human PSC cultures progress through a three-stage differentiation process to generate human intestinal organoids. By day 3 of the protocol, cultures exhibit characteristics typical of definitive endoderm and mid-/hindgut differentiation is initiated. During mid-/hindgut differentiation (days 5 - 9), cells form mid-/hindgut spheroids that are released from the cell monolayer into the culture medium. These spheroids are collected and embedded in extracellular matrix. (B) Embedded mid-/hindgut spheroids cultured in STEMdiff™ Intestinal Organoid Growth Medium mature into intestinal organoids (days in parentheses indicate days post-embedding in a given passage). Once established, intestinal organoids can be maintained and expanded in culture by passaging every 7 - 10 days. After multiple passages, the organoids generally exhibit less sinking within the matrix dome and a lower proportion of mesenchymal cells.

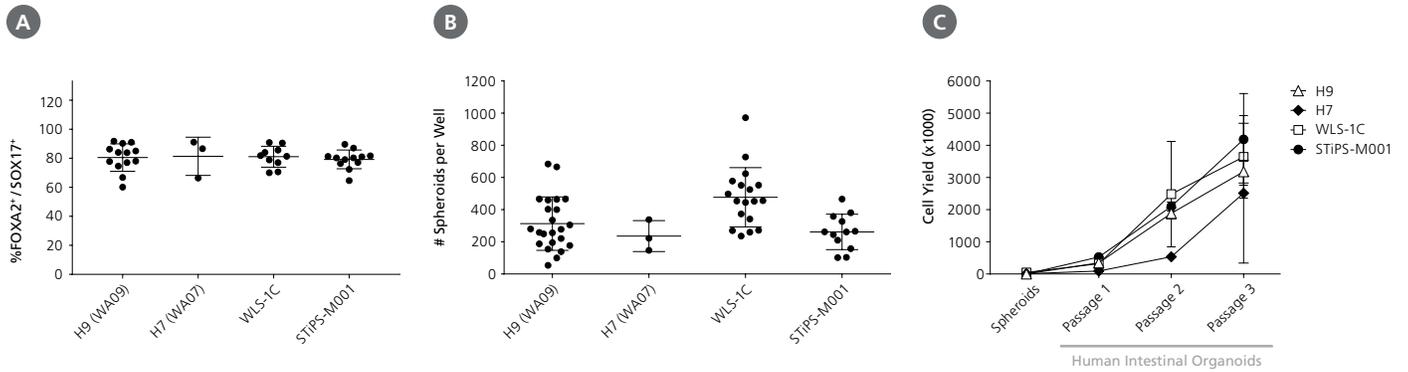


Figure 12. STEMdiff™ Intestinal Organoid Kit Supports Robust Differentiation and Expansion Across ESC and iPSC Lines

STEMdiff™ Intestinal Organoid Kit enables high-efficiency generation of intestinal organoids from both ES cells (H9, H7) and iPSC cells (WLS-1C, STIPS-M001). (A) Organoids initiated from a variety of cell lines show efficient induction of definitive endoderm, measured by co-expression of FOXA2 and SOX17 on day 3 of differentiation. (B) Both ES and iPSC cell-derived cultures demonstrate efficient spheroid formation upon mid-/hindgut induction. The total number of spheroids obtained per well in a given differentiation is shown. (C) Organoids cultured from either ES or iPSC cells can be expanded and maintained over multiple passages. Shown is the total cell yield per passage. Organoids were passaged every 7 - 10 days with a split ratio between 1 in 2 and 1 in 4. Data points represent the mean of 3 biological replicates. Error bars throughout represent standard deviation of the mean.

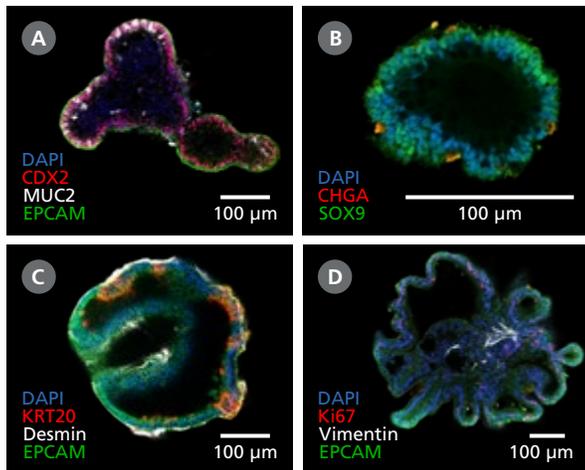


Figure 13. Characteristics of Intestinal Organoids Cultured Using STEMdiff™ Intestinal Organoid Growth Medium

Intestinal organoids were stained and examined for marker expression by immunocytochemistry. (A,B) Intestinal organoids express markers of intestinal progenitor cells including CDX2 and the intestinal crypt marker SOX9. The organoids incorporate a polarized epithelium, visualized by the localization of EPCAM to the exterior (basolateral) surface of the organoids (A), and express markers typical of mature cell types including MUC2 (goblet cells, A) and CHGA (enteroendocrine cells, B). (C,D) Observation of desmin (C) and vimentin (D) in intestinal organoids demonstrates incorporation of mesenchymal cells in the organoid cultures, while KRT20 (C) and Ki67 (D) are markers of differentiated intestinal cells and actively dividing progenitor cells, respectively. Images are digital cross-sections of whole-mount imaging of intact organoids.

Hepatic Organoids

The development of hepatic organoid culture techniques has provided the hepatic research field with a convenient method for sustained maintenance of liver cells in vitro.^{4,5} Hepatic progenitor organoids are cultured by isolating and expanding liver stem and progenitor cells, which are postulated to reside in hepatic ducts. Culture of hepatic progenitor cells as organoids produces spherical organoids consisting primarily of progenitor cells that can be further differentiated to either hepatocytes or cholangiocytes.⁶

HepatiCult™ Organoid Growth Medium (Mouse)

Cell Culture Medium for Establishment and Maintenance of Mouse Hepatic Organoids

HepatiCult™ Organoid Growth Medium (Mouse) is a serum-free medium that allows for rapid generation of hepatic progenitor organoids from mouse liver tissue. Organoid culture may be initiated from hepatic ducts, duct fragments, single cells, or cryopreserved organoids and cultured in Corning® Matrigel® domes or as a dilute Matrigel® suspension.

Why Use HepatiCult™ Organoid Growth Medium (Mouse)?

CONVENIENT. In vitro system for generating organoids within a week.

STEP-BY-STEP PROTOCOL. No injury models, hand-picking of ducts, or cell sorting required.

SIMPLE, TWO-COMPONENT FORMAT. Serum-free medium formulation.

FLEXIBLE PROTOCOL. Organoids be can grown from duct fragments or single cells and cultured in matrix domes or suspension.

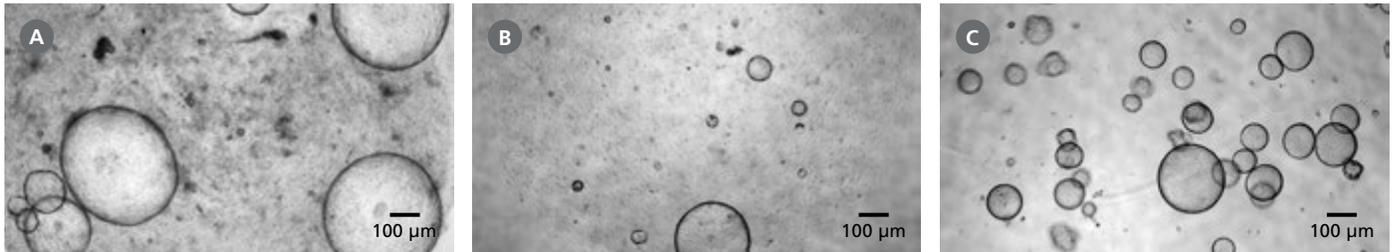


Figure 14. Mouse Hepatic Progenitor Organoids Can Be Initiated from a Variety of Starting Materials

HepatiCult™ Organoid Growth Medium (Mouse) enables the initiation of hepatic progenitor organoids from (A) duct fragments, (B) single cells or (C) cryopreserved organoids. All organoids were grown in Matrigel® domes and imaged on day 7 of primary culture or the first passage post thaw (cryopreserved organoids).

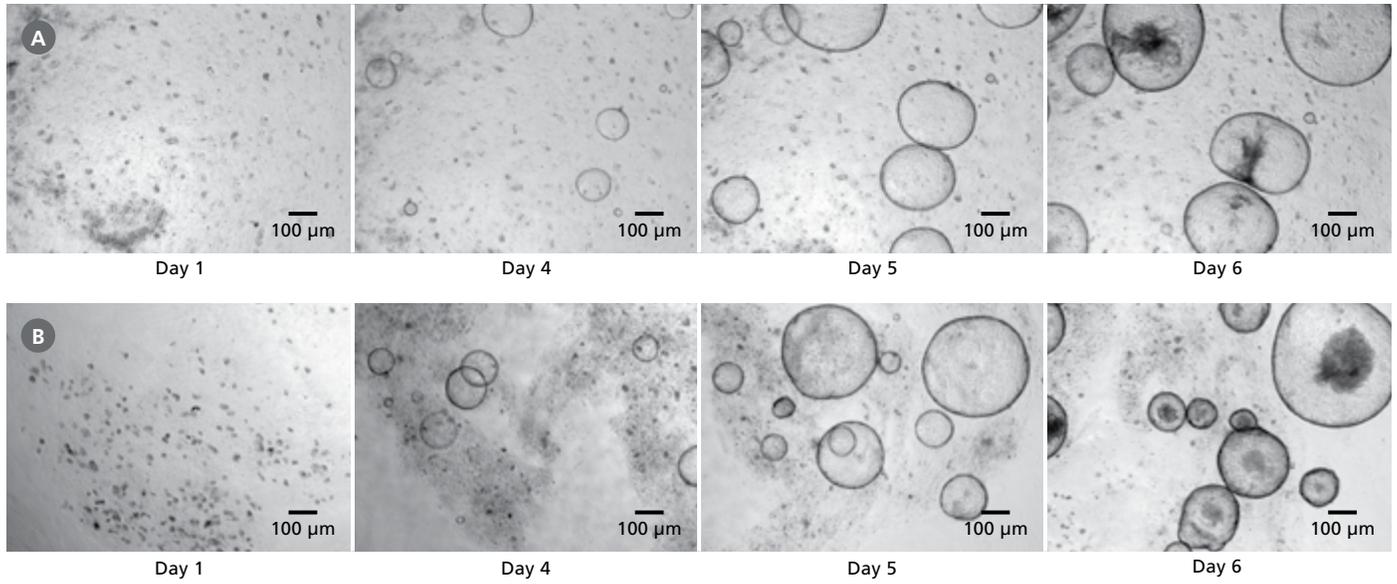


Figure 15. Hepatic Organoids Can Be Grown in Matrigel® Domes or in a Dilute Matrigel® Suspension

Hepatic progenitor organoids cultured from freshly isolated mouse hepatic duct fragments in HepatiCult™ Organoid Growth Medium (Mouse) can be plated in (A) Matrigel® domes or (B) a dilute Matrigel® suspension. Organoids grown in either culture condition are ready for passage within 4-7 days.

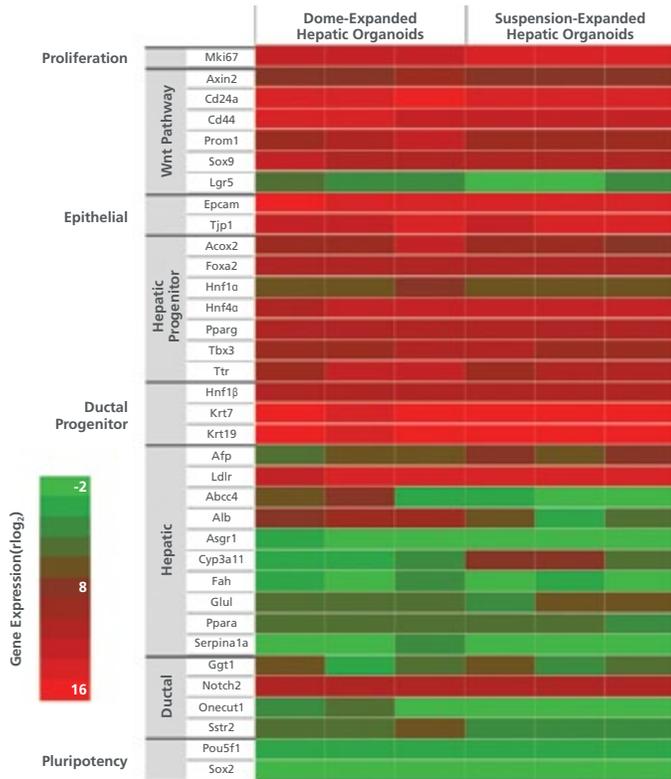


Figure 16. Analysis of Organoid Gene Expression

Analysis of marker expression by RNA-seq shows organoids grown in HepatiCult™ Organoid Growth Medium (Mouse) in either Matrigel® domes or in a dilute Matrigel® suspension express markers associated with hepatic stem and progenitor cells. The organoids also express low levels of genes associated with mature hepatic cell types, including cholangiocytes and hepatocytes. Columns represent biological replicates at passages ranging from passage 1 - 40.

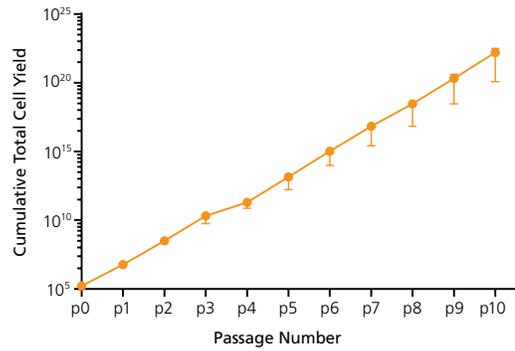


Figure 17. Expansion of Organoids Grown in HepatiCult™ Organoid Growth Medium (Mouse)

Organoids cultured with HepatiCult™ Organoid Growth Medium (Mouse) show efficient growth over multiple passages. Cultures were split with an average split ratio of 1:25 at each passage.

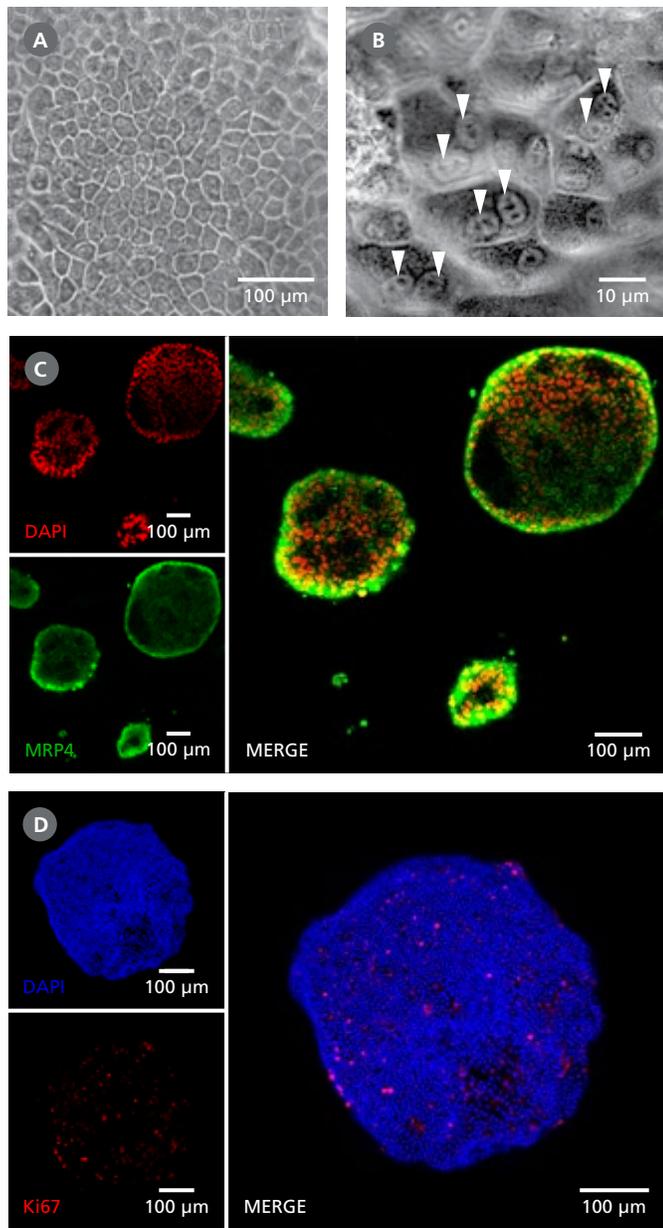


Figure 18. Organoids Grown in HepatiCult™ Organoid Growth Medium (Mouse) Display Some Characteristics Typical of the Mature Hepatic Epithelium

(A) Hepatic progenitor organoids exhibit the polygonal morphology typical of the hepatic epithelium. (B) Hepatic progenitor organoids show binucleation (arrows), a common feature of mature hepatocytes. (C) Immunocytochemistry analysis shows localization of MRP4 (green), a membrane-bound, unidirectional efflux transporter, along the exterior of the organoids and DAPI (red) localized to the cellular nuclei. This indicates cellular polarization of the organoids with the basolateral surface of the epithelium distal from the lumen. (D) Hepatic organoids contain an actively dividing progenitor population, shown by the expression of Ki67 (red). Cell nuclei are stained with DAPI (blue).

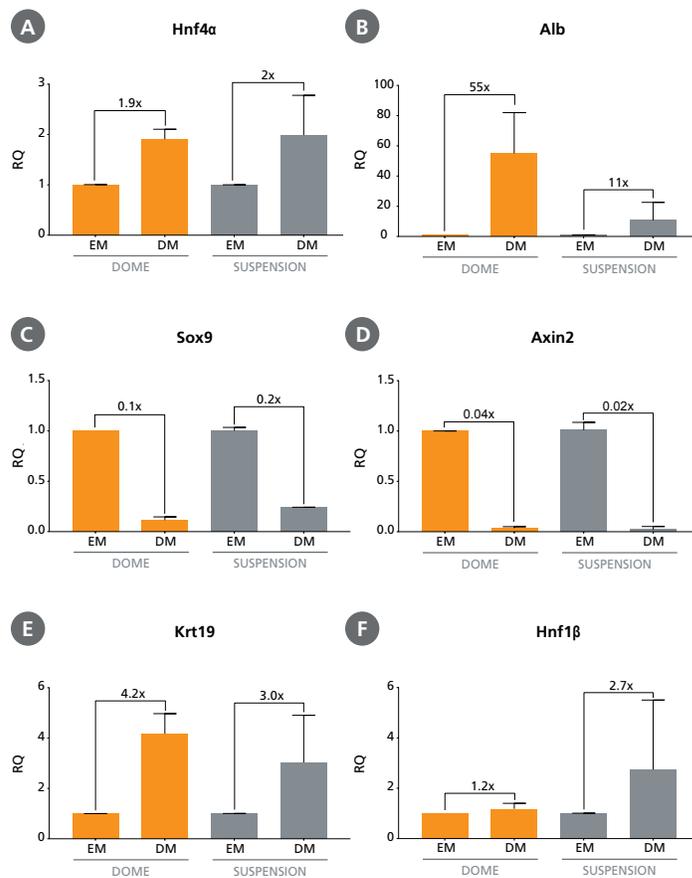


Figure 19. Differentiation of Hepatic Progenitor Organoids

Hepatic progenitor organoids grown in HepatiCult™ Organoid Growth Medium (Mouse) (EM) can be differentiated to resemble more mature cell types when switched to a differentiation medium (DM). After switching to published differentiation medium, hepatic organoids show the upregulation of mature hepatic markers including (A) Hnf4a and (B) Alb, downregulation of hepatic stem cell and progenitor markers (C) Sox9 and (D) Axin2 and upregulation of ductal markers (E) Krt19 and (F) Hnf1β. Relative quantification (RQ) for each marker is reported relative to 18S and TBP housekeeping genes and normalized with respect to hepatic progenitor organoids grown in HepatiCult™ Organoid Growth Medium (Mouse) and cultured in Matrigel® domes.

Pancreatic Organoids

Pancreatic organoids are cultured through isolation and expansion of pancreatic stem and progenitor cells from pancreatic ducts.⁷ When cultured with the appropriate growth factors and extracellular matrix, pancreatic progenitor cells form organoids resembling the exocrine component of the pancreas. In addition, isolation of primary tissue from mouse primary tumors and metastases allows for the growth of tumor-derived organoids, providing a model for pancreatic carcinomas and pancreatic ductal adenocarcinoma progression. These organoids retain key characteristics of the parental tumor, including genotype and phenotype, providing a convenient model system for in vitro experimentation.

PancreaCult™ Organoid Growth Medium (Mouse)

Cell Culture Medium for Establishment and Maintenance of Mouse Pancreatic Exocrine Organoids

PancreaCult™ Organoid Growth Medium (Mouse) enables the growth of pancreatic exocrine organoids from pancreatic ducts, duct fragments, single cells, or organoid fragments. Pancreatic organoids can be maintained through extended passaging or cryopreservation, providing a readily available source of cells for future experiments.

Why Use PancreaCult™ Organoid Growth Medium (Mouse)?

CONVENIENT. In vitro system for generating organoids within a week.

STEP-BY-STEP PROTOCOL. No injury models, hand-picking of ducts, or cell sorting required.

SIMPLE, TWO-COMPONENT FORMAT. Serum-free medium formulation.

FLEXIBLE PROTOCOL. Organoids can be grown from duct fragments or single cells and cultured in matrix domes or suspension.

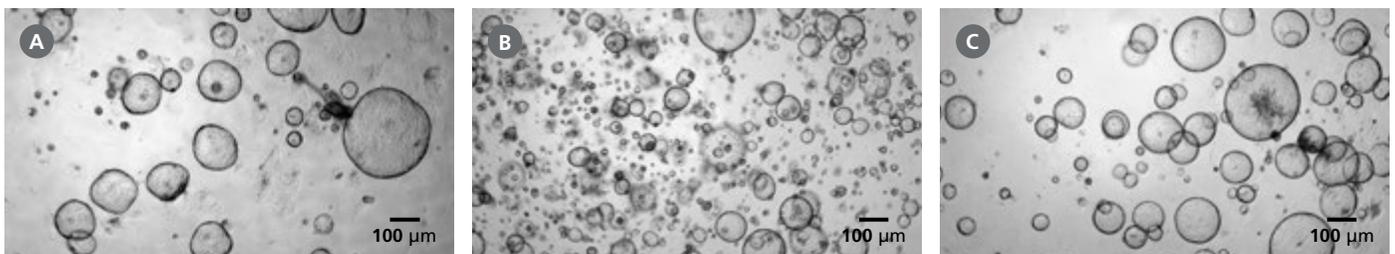


Figure 20. Mouse Pancreatic Organoids Initiated from a Variety of Starting Materials

PancreaCult™ Organoid Growth Medium (Mouse) enables the initiation of pancreatic exocrine organoids from (A) duct fragments, (B) single cells, and (C) cryopreserved organoid fragments. All organoids were grown in Matrigel® domes. Organoids were imaged on day 4 or day 5 of primary culture (duct fragments and single cells, respectively) or day 3 of the first passage post-thaw (cryopreserved organoids).

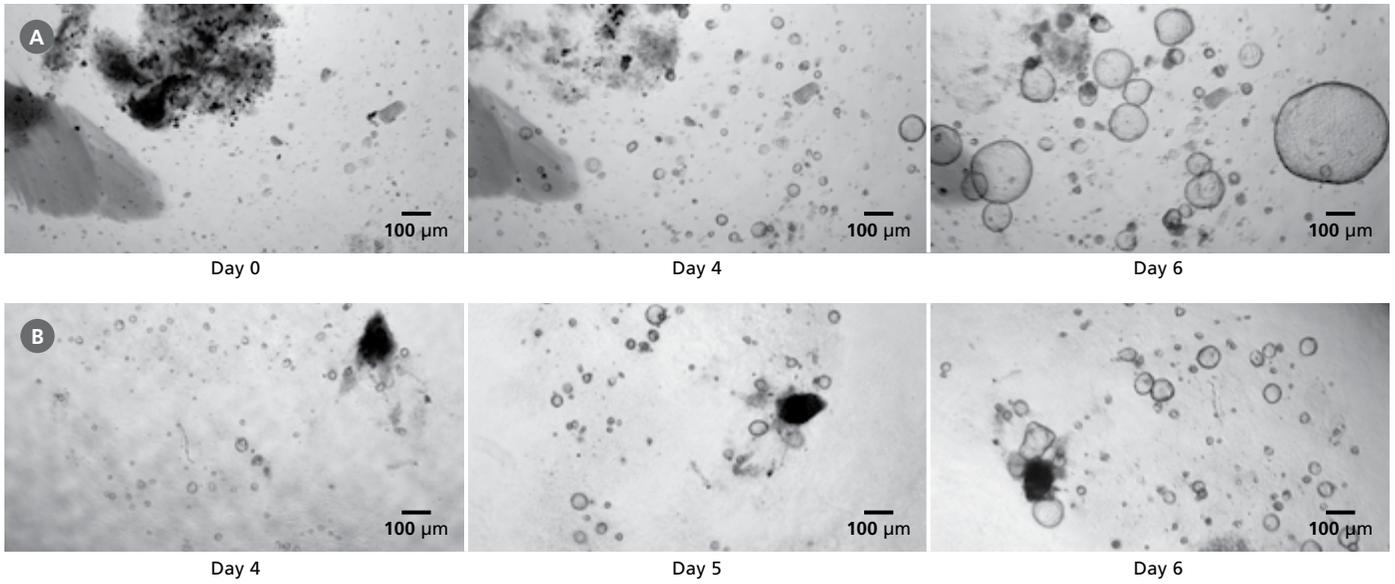


Figure 21. Pancreatic Organoids Grown in Matrigel® Domes or as a Dilute Matrigel® Suspension

Organoids cultured using PancreaCult™ Organoid Growth Medium (Mouse) from freshly isolated pancreatic tissue fragments and plated in (A) Matrigel® domes or (B) as a dilute Matrigel® suspension. Organoids grown in either culture condition are typically ready for passage within 3 - 6 days.

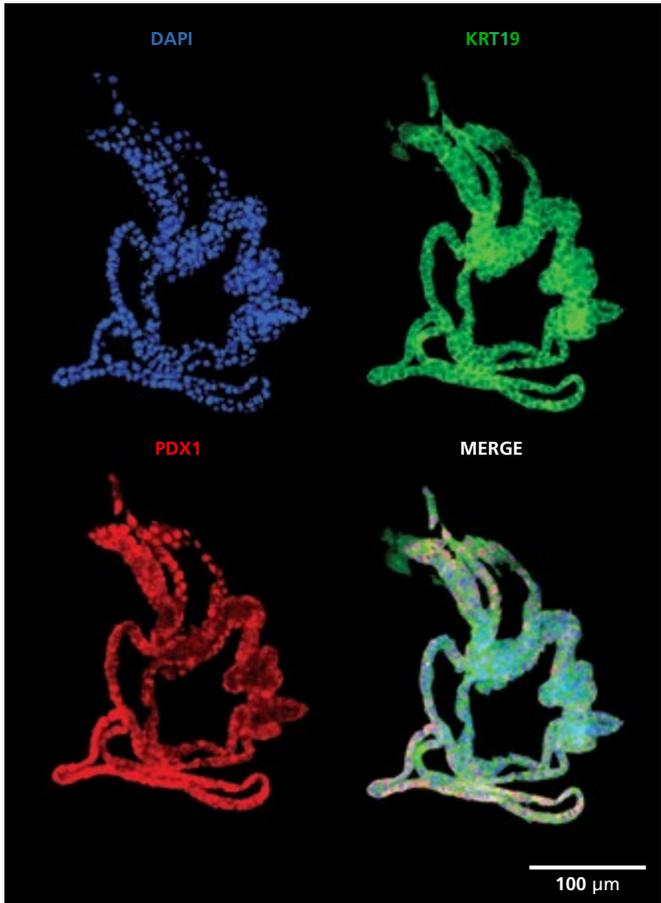


Figure 22. Pancreatic Exocrine Organoids Display Markers of Pancreatic Progenitor and Ductal Cells

Pancreatic exocrine organoids grown in PancreaCult™ Organoid Growth Medium (Mouse) and stained for nuclei (DAPI, blue), ductal marker KRT19 (green) and pancreatic progenitor marker PDX1 (red). Organoids were imaged during passage 12 on day 5.

Note: The folded appearance of epithelium is a function of cryosectioning and not representative of the shape of proliferating organoids.

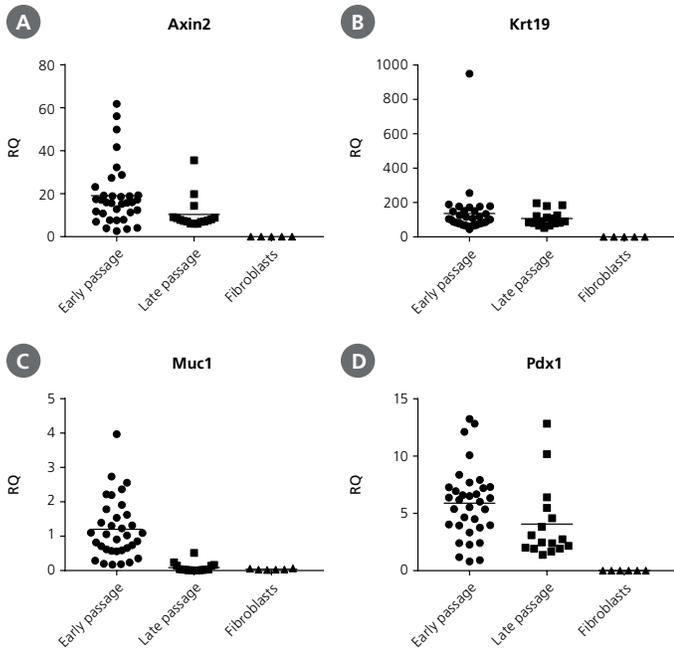


Figure 23. Pancreatic Exocrine Organoids Retain Pancreatic Marker Expression During Passaging

Pancreatic organoids express stem cell markers and those typical of the pancreatic exocrine system, including (A) Axin2, (B) Krt19, (C) Muc1, and (D) Pdx1. Relative quantification (RQ) of each marker is reported relative to the 18S and TBP housekeeping genes and normalized to C57/Bl6 pancreatic tissue. Marker expression was assayed during early passages (passage 1-5) and late passages (passage 6-10).

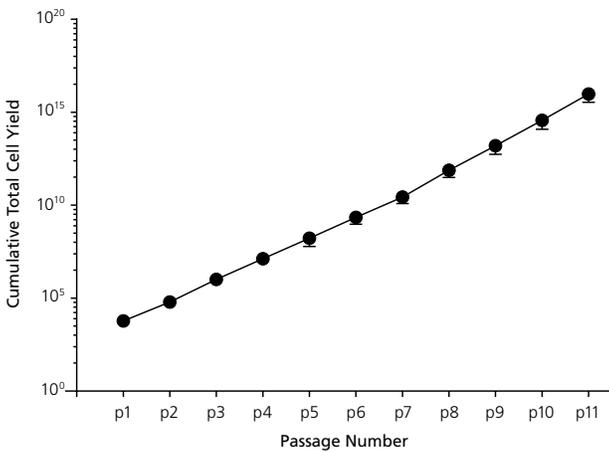


Figure 24. Expansion of Organoids Grown in PancreaCult™ Organoid Growth Medium (Mouse)

Organoids cultured with PancreaCult™ Organoid Growth Medium (Mouse) show efficient growth over multiple passages. Cultures were split with an average split ratio of 1:16 at each passage.

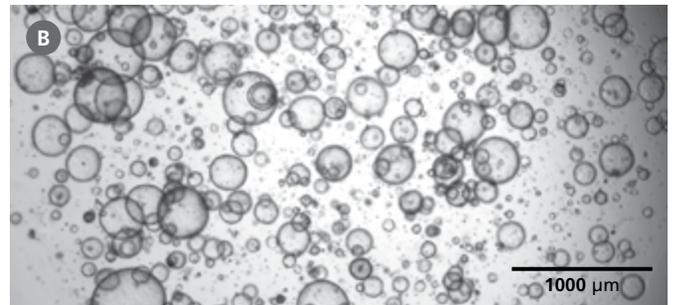
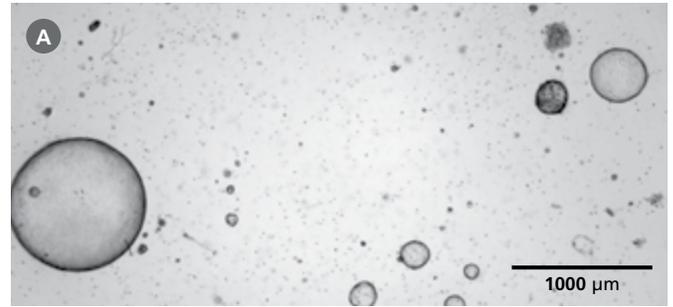


Figure 25. Pancreatic Exocrine Organoids Provide a Model for Pancreatic Carcinomas

PancreaCult™ Organoid Growth Medium (Mouse) supports the growth of organoids from pancreatic carcinomas. Pancreatic ducts were isolated from KPC mice (*Kras*^{+LSL-G12D}, *Trp53*^{+LSL-R172H}; *Pdx1*-Cre) and cultured in PancreaCult™ Organoid Growth Medium (Mouse). Organoids were imaged on (A) day 4 of primary culture and (B) day 4 after the first passage. An activated KRAS genotype was retained in organoids during culture. Data used with permission from Dr. David Tuveson.

Kidney Organoids

The kidney plays an important physiological role in the body, such that nephrotoxicity is a critical consideration during drug screening and development. Despite this, the limited availability of relevant in vitro models has presented challenges for studying kidney health and function. Kidney organoids are addressing this challenge by providing efficient in vitro culture systems that mimic the structure and cellular architecture of the nephron and associated mesenchyme and endothelium.⁸ These kidney organoids are composed of convoluted tubular structures with typical nephron-like segmentation marked by the expression of podocyte (podocalyxin [PODXL]), proximal (lotus tetragonolobus lectin [LTL]), and distal tubule (E-cadherin [ECAD]) markers.

STEMdiff™ Kidney Organoid Kit

Serum-Free Medium Kit for the Culture of Kidney Organoids from PSCs

STEMdiff™ Kidney Organoid Kit is a complete, serum-free cell culture medium system that supports highly efficient and reproducible generation of human pluripotent stem cell (PSC)-derived kidney organoids in a simple two-stage differentiation protocol. It has an optimized, quality controlled formulation to increase reproducibility and efficiency across multiple pluripotent cell lines. This kit has been optimized for the differentiation of PSCs previously cultured in mTeSR™1 (Catalog #85850).

Why Use the STEMdiff™ Kidney Organoid Kit?

RELEVANT. Enables generation of human kidney organoids that model the developing nephron and associated endothelium and mesenchyme.

SIMPLE. Minimizes culture manipulations with a two-stage culture system and easy-to-follow protocol.

RELIABLE. Provides low experimental variability through an optimized formulation and rigorous quality controls.

HIGH THROUGHPUT. Generates organoids in 96- and 384-well formats.

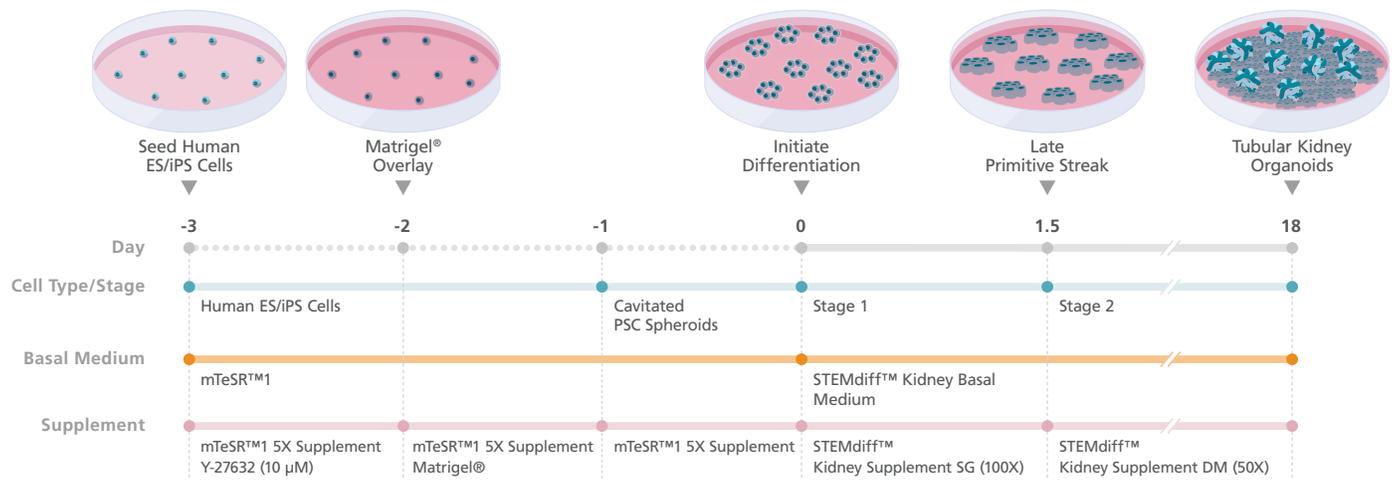


Figure 26. Differentiation of PSCs to Human Kidney Organoids with the STEMdiff™ Kidney Organoid Kit

Human pluripotent stem cell cultures progress through a simple two-stage process to generate kidney organoids. hPSCs are plated and overlaid with Corning® Matrigel® to form cavitated spheroids. These are induced towards the late primitive streak, posterior intermediate mesoderm, and metanephric mesenchyme and form tubular kidney organoids by day 18 of differentiation.

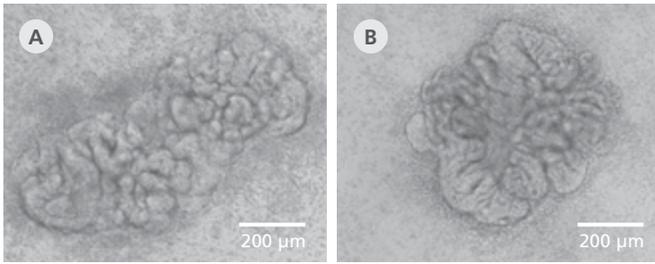


Figure 27. Representative Images of Kidney Organoids

The STEMdiff™ Kidney Organoid Kit facilitates the directed differentiation of PSCs to form kidney organoids that model the developing nephron. Pictured are human kidney organoids grown using the STEMdiff™ Kidney Organoid Kit differentiated from (A) iPS (WLS-1C) or (B) ES (H9) cells and imaged on day 12 and day 18 of differentiation, respectively.

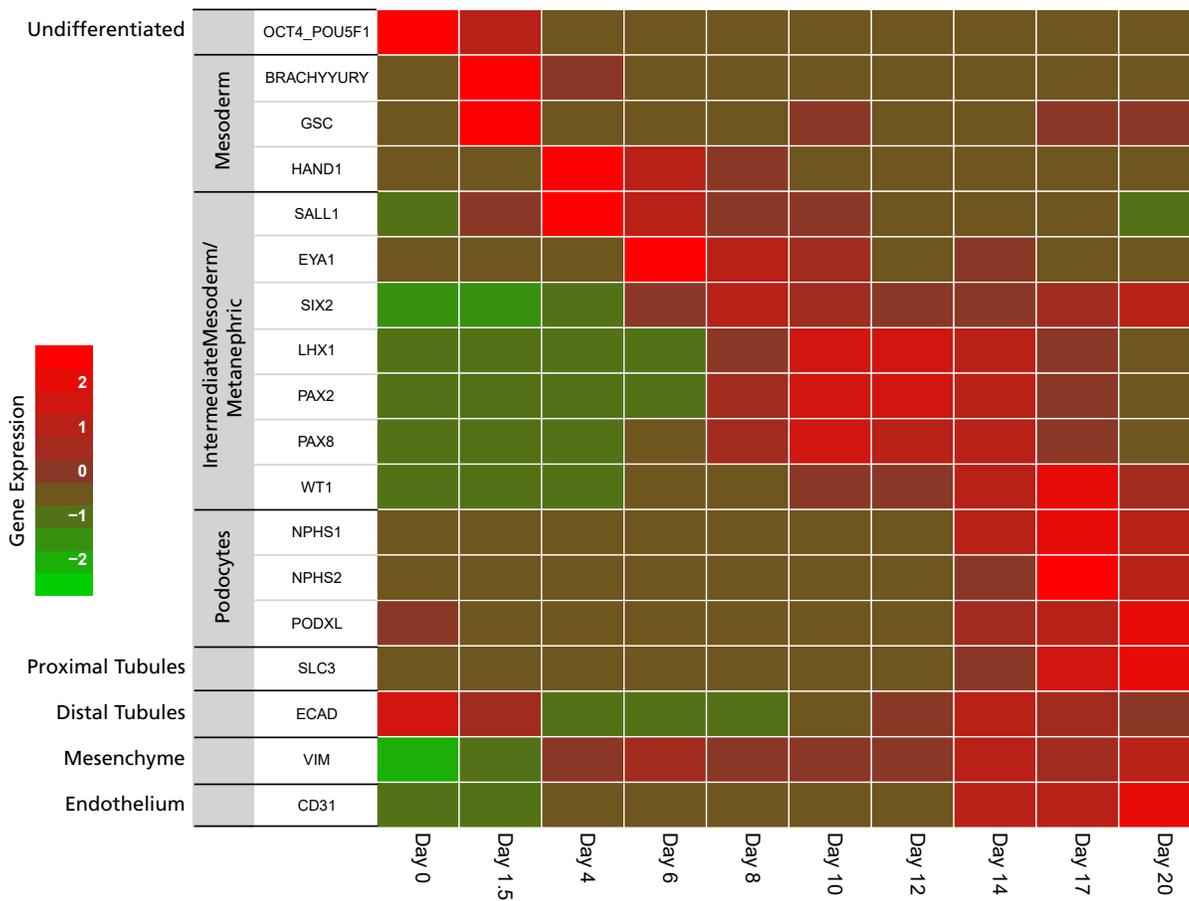
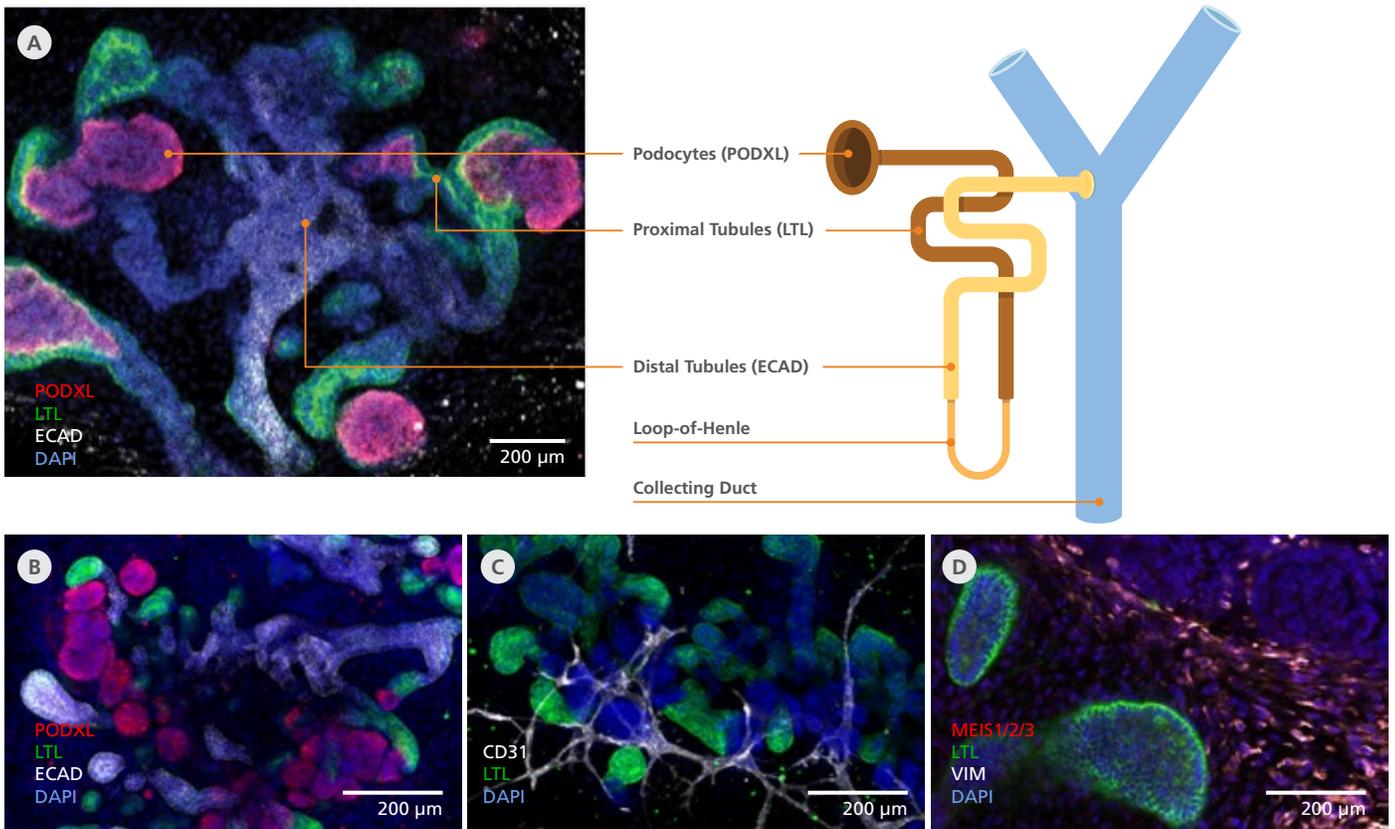
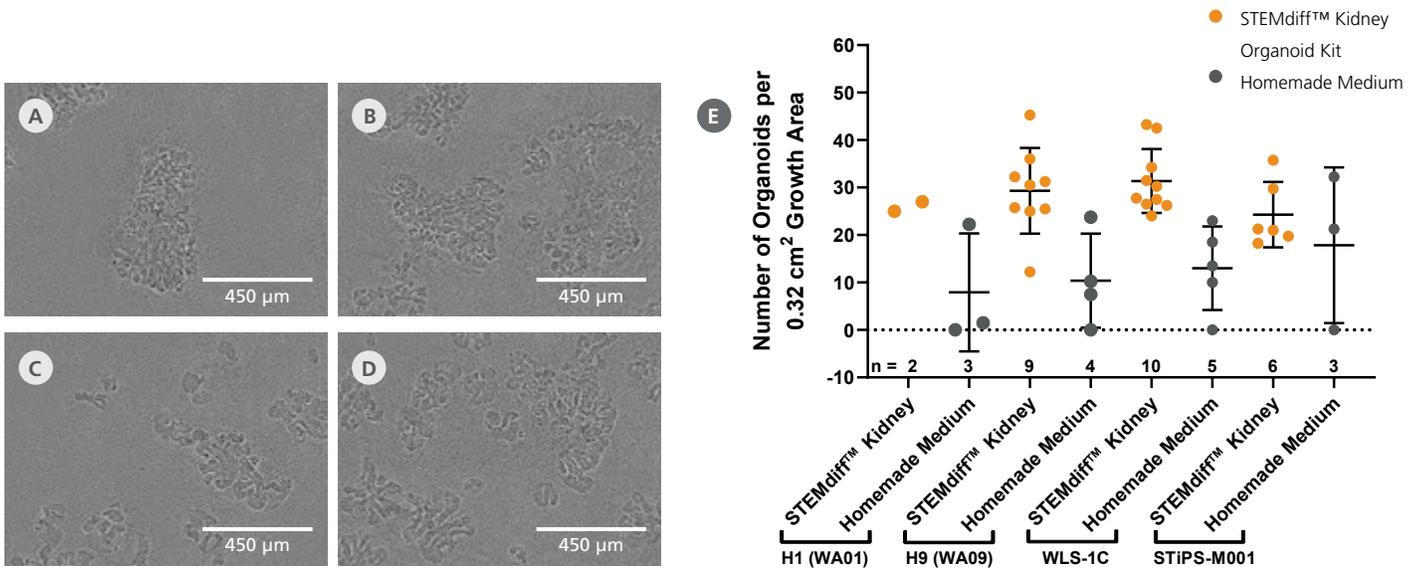


Figure 28. Kidney Organoids Grown Using the STEMdiff™ Kidney Organoid Kit Show the Expected Changes in Gene Expression During Differentiation

As organoids progress through the stages of differentiation to more mature renal cell types, gene expression shifts from markers of pluripotency (day 0) to the mesoderm (day 1.5 - 4) and to the intermediate mesoderm/metanephric mesenchyme by day 4 - 12. Markers of the podocytes, proximal and distal tubules, mesenchyme and endothelium are observable starting by day 14. Marker levels were assessed in four independent experiments by RT-qPCR and normalized to expression levels of undifferentiated cells.



Airway Organoids

Three-dimensional human airway organoids can be generated by differentiation of human airway epithelial cells. Fully differentiated human airway organoids represent a versatile and physiologically relevant organotypic model system for studying the airway epithelium, consisting of basal cells, ciliated cells, club cells, and goblet cells. Airway organoid cultures can be used for in vitro study of the cell biology of the respiratory epithelium, and to understand the underlying mechanisms of chronic respiratory diseases such as cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), asthma, idiopathic pulmonary fibrosis (IPF), lung cancer, and infectious diseases. Furthermore, airway organoids can also be used for high-throughput screening of efficacy and toxicity of compounds.

PneumaCult™ Airway Organoid Kit

Cell Culture Medium Kit for Generation of Human Airway Organoids

PneumaCult™ Airway Organoid Kit is a novel, serum-free medium kit that supports the efficient generation of fully differentiated and functional airway organoids from both healthy and disease samples. Airway organoid cultures provide an alternative method to air-liquid interface (ALI)-based cultures for in vitro human airway modeling. Since this culture system does not require the use of cell culture inserts, it is amenable to high-throughput drug screening and can be used in large-scale screening for CF transmembrane conductance regulator (CFTR) modulators.

PneumaCult™ Airway Organoid Kit provides the necessary components to prepare PneumaCult™ Airway Organoid Seeding Medium, that allows for initiation of 3D organoid culture, and PneumaCult™ Airway Organoid Differentiation Medium, to further obtain morphologically representative and fully differentiated human airway organoids.

Why Use PneumaCult™ Airway Organoid Kit?

PHYSIOLOGICAL. Three-dimensional in vitro system recapitulates the in vivo human airway.

OPTIMIZED. Complete system for expansion and differentiation of human airway epithelial cells, compatible with PneumaCult™-Ex Plus.

RELIABLE. Rigorous raw material screening and extensive quality control testing ensure reproducibility and minimal lot-to-lot variability.

USER-FRIENDLY. Convenient format and easy-to-use protocol.

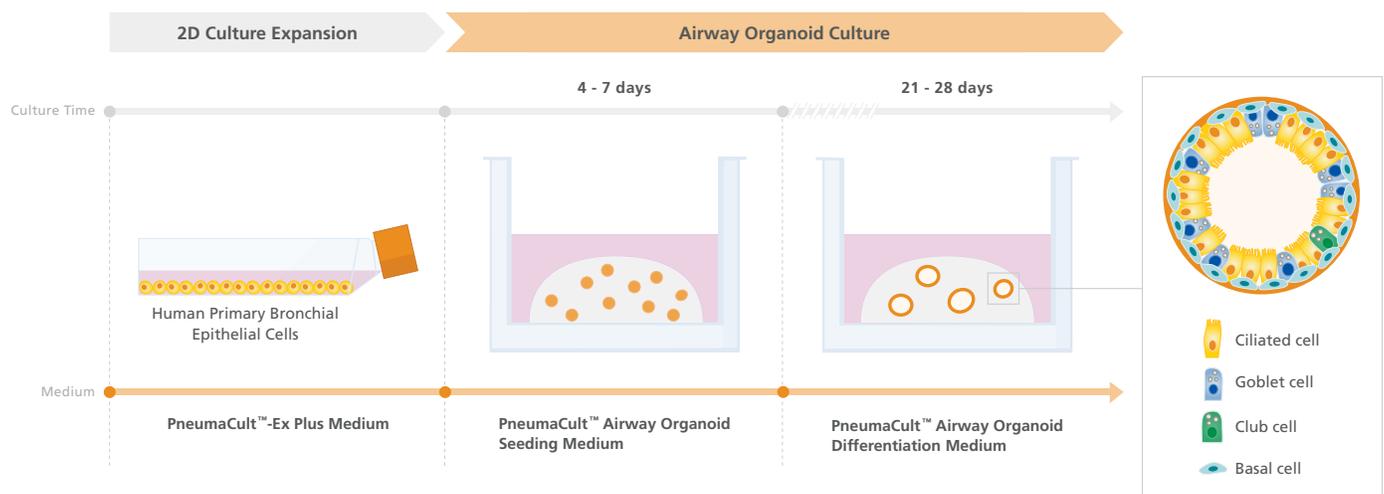


Figure 31. Schematic of Human Airway Organoid Culture Workflow

In the early two-dimensional expansion phase of the human airway organoid culture procedure, human airway epithelial cells (HAECs) are expanded using PneumaCult™-Ex Plus Medium. The HAECs are then embedded into a Matrigel® dome and expanded for 4 - 7 days using PneumaCult™ Airway Organoid Seeding Medium. Following the expansion, the HAECs are differentiated using PneumaCult™ Airway Organoid Differentiation Medium for an additional 21+ days.

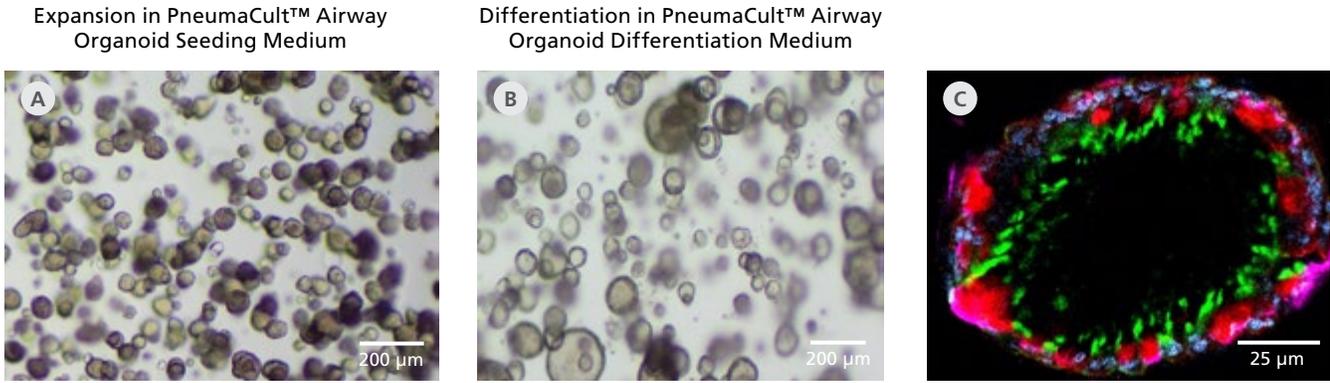


Figure 32. Fully Differentiated Human Airway Organoids Generated Using PneumaCult™ Airway Organoid Kit

(A) Bright-field image of airway organoids growing in PneumaCult™ Airway Organoid Seeding Medium at day 7 exhibit basal cell spheroid morphology. (B) Bright-field image of airway organoids differentiated in PneumaCult™ Airway Organoid Differentiation Medium at day 21 exhibit hollow lumens. (C) Airway organoid stained for ZO-1 (junction protein marker, red), MUC5AC (goblet cell marker, purple), AC-Tubulin (ciliated cell marker, green), and DAPI (nuclei, blue).

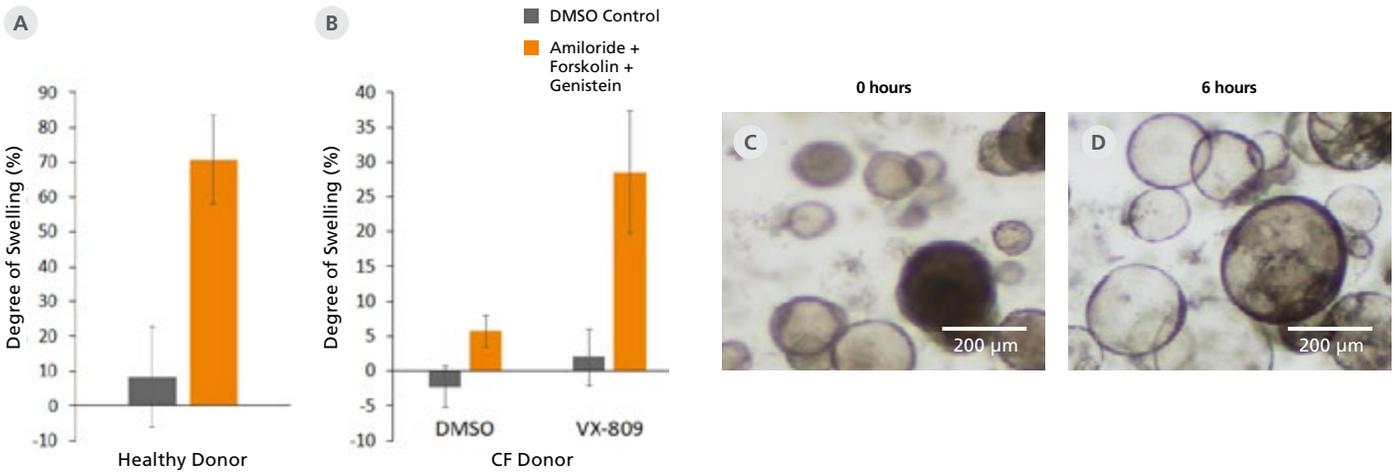


Figure 33. Forskolin-Induced Swelling of Airway Organoids

(A) Forskolin-treated organoids derived from healthy donors increased in size compared to the DMSO control, indicating functional CFTR protein expression. (B) Forskolin-induced swelling is lost in organoids derived from CF donors, but re-established in VX-809-treated airway organoids. Error bars represent \pm 95% confidence interval for the mean ($n=3$). Bright-field images of airway organoids taken during the Forskolin swelling assay at (C) 0 hours and (D) 6 hours show organoid swelling after treatment.

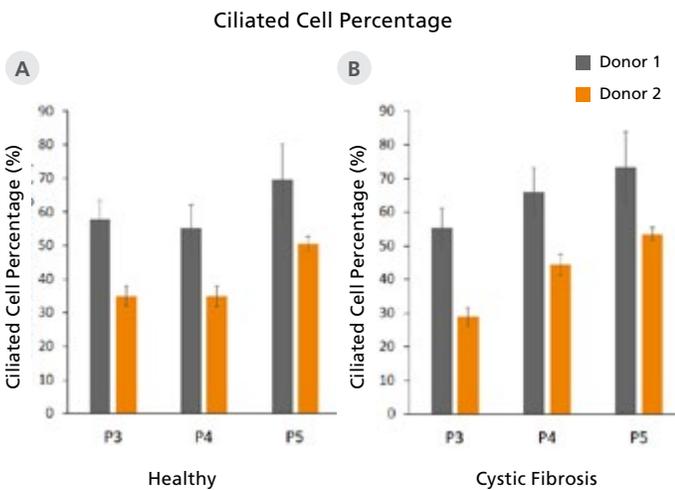


Figure 34. Fully Differentiated Airway Organoids Retain Morphological Characteristics at Different Passages

The ciliated cell percentage in organoids grown from (A) healthy and (B) CF donors using PneumaCult™ Airway Organoid Kit increased from P3 to P5. The total and ciliated cells were counted using a hemocytometer. Error bars represent \pm 95% confidence interval for the mean ($n=3$).

Cerebral Organoids

Differentiation of cerebral organoids from PSCs generates three-dimensional in vitro cultures that recapitulate the developmental processes and organization of the developing human brain. These “mini-brains” provide unique, physiologically relevant in vitro model systems for the study of human neurological development and disease. Cerebral organoids have important applications in studying human brain development and neurological disorders such as autism, schizophrenia, or brain defects caused by Zika virus infection.

STEMdiff™ Cerebral Organoid Kit

Cell Culture Medium Kit for Establishment and Maturation of Human Cerebral Organoids

Based on the formulation published by Lancaster and Knoblich,⁸ the STEMdiff™ Cerebral Organoid Kit is a serum-free culture system that is designed to generate cerebral organoids from human ES or iPS cells. Large, optically dense organoids that display multiple cortical-like regions when cryosectioned and immunostained are observed in 40 days, providing relevant models for the early developing human cortex.

Why Use STEMdiff™ Cerebral Organoid Kit?

PHYSIOLOGICAL. Three-dimensional in vitro system recapitulates the developmental processes and organization of the developing brain.

INNOVATIVE. Serum-free human pluripotent stem cell-based model enables the study of development and disease processes.

OPTIMIZED. Formulation is optimized for increased efficiency of organoid formation.

RELIABLE. Rigorous raw material screening and extensive quality control testing ensure reproducibility and minimal lot-to-lot variability.

SIMPLE. Convenient format and easy-to-use protocol.

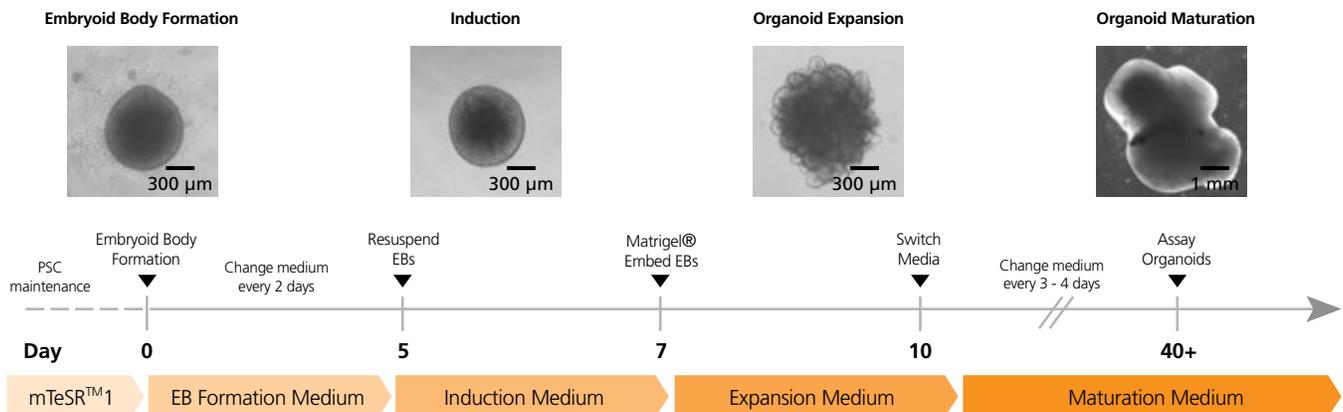


Figure 35. Schematic for the STEMdiff™ Cerebral Organoid Kit

Human pluripotent stem cells (either embryonic or induced pluripotent stem cells) maintained in mTeSR1™ were dissociated into single-cell suspensions using Gentle Cell Dissociation Reagent (GCDR) and seeded at a density of 9,000 cells/well in a U-Bottom 96-well Ultra-Low Attachment Plate (Corning®) in embryoid body (EB) Formation Medium + 10 µM Rho-Kinase Inhibitor (ROCKi). EBs were fed every 2 days with EB Formation Medium without ROCKi. After 5 days, EBs were transferred to Induction Medium in a 24-well Ultra-Low Attachment Plate (Corning®). EBs were cultured for an additional 2 days and were then embedded in liquid Matrigel® (Growth Factor Reduced, Corning®). They were then transferred to a non-tissue culture treated 6-well plate (12 -16 organoids/well). Embedded organoids were maintained in Expansion Medium for 3 days. On Day 10, organoids were switched to Maturation Medium and cultured on an orbital shaker set at 57-95 RPM (Infors HT). Organoids were fed every 3 - 4 days with Maturation Medium. On Day 40, organoids were processed for analysis by RT-qPCR or immunostaining followed by cryosectioning.

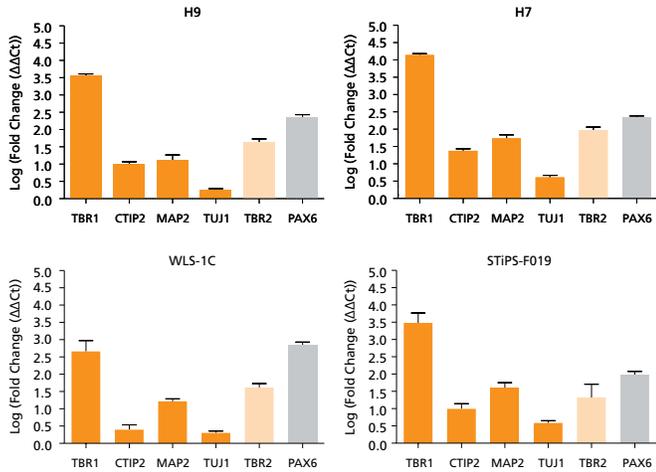


Figure 36. Day 40 Cerebral Organoids Contain Progenitor and Neuron Populations That Organize into Distinct Layers

RT-qPCR analysis showed upregulation of both neural progenitor and mature neuron transcripts as Log(Fold Change ($\Delta\Delta Ct$)) (Average \pm SEM n = 2 per cell line, ≥ 3 organoids per analysis). Data were normalized to 18S/TBP and compared to undifferentiated PSC control.

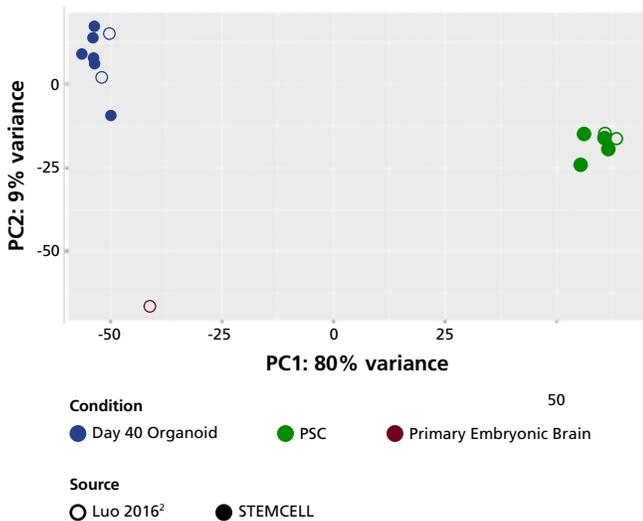


Figure 37. Organoids Generated with STEMdiff™ Cerebral Organoid Kit Are Similar to Organoids Produced in Literature

Principal component analysis of PSC and cerebral organoid transcriptomes. Cerebral organoids generated using the STEMdiff™ Cerebral Organoid Kit (filled blue circles) clustered together, and clustered with previously published cerebral organoids (open blue circles). The first principal component accounted for the majority of variance observed (PC1; 80%) and distinguished the cerebral organoid samples from the PSCs (green circles). The second principal component accounted for only 9% of the variation, and highlighted the modest difference in gene expression between cultured organoids and primary embryonic fetal brain samples (19 post-conceptual weeks, brown circles).

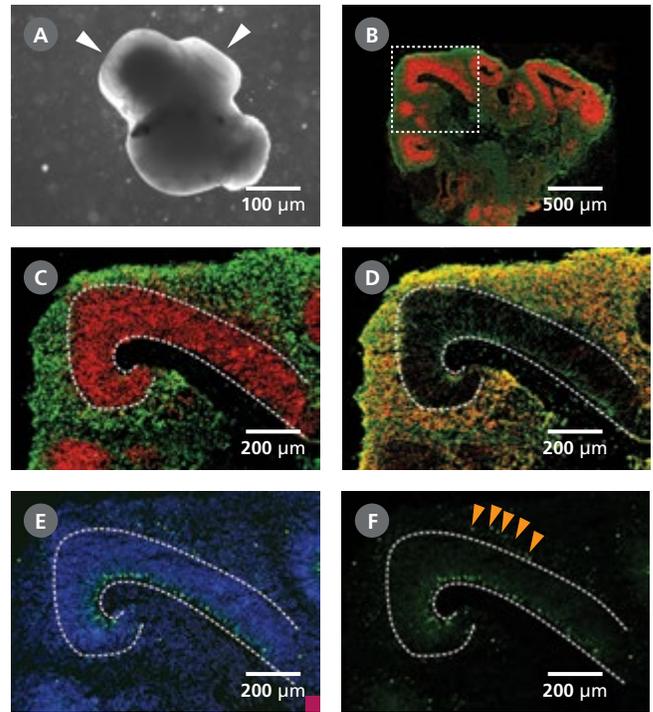


Figure 38. Characterization of Cerebral Organoids Generated Using the STEMdiff™ Cerebral Organoid Kit

(A) A representative phase-contrast image of a whole cerebral organoid at Day 40 generated using the STEMdiff™ Cerebral Organoid Kit. Cerebral organoids at this stage are made up of phase-dark structures that may be surrounded by regions of thinner, more translucent structures that display layering (arrowheads). (B) Immunohistological analysis on cryosections of cerebral organoids reveals cortical regions within the organoid labeled by the apical progenitor marker PAX6 (red) and neuronal marker class III β -tubulin (TUJ-1) (green). (C-F) Inset of boxed region from (B). (C) PAX6+ apical progenitors (red, enclosed by dotted line) are localized to a ventricular zone-like region. Class III β -tubulin+ neurons (green) are adjacent to the ventricular zone. (D) CTIP2, a marker of the developing cortical plate, co-localizes with class III β -tubulin+ neurons in a cortical plate-like region. Organization of the layers recapitulates early corticogenesis observed during human brain development. (E) Proliferating progenitor cells labeled by Ki-67 (green) localize along the ventricle, nuclei are counterstained with DAPI (blue). (F) An additional population of Ki-67+ cells is found in an outer subventricular zone-like region (arrowheads).

Product Information

PRODUCT	SIZE	CATALOG #
IntestiCult™ Organoid Growth Medium (Mouse)	100 mL	06005
Mouse Intestinal Organoids	200 Organoids	70931
IntestiCult™ Organoid Growth Medium (Human)	100 mL	06010
IntestiCult™ Organoid Differentiation Medium	100 mL	100-0214
STEMdiff™ Intestinal Organoid Kit	1 Kit	05140
STEMdiff™ Intestinal Organoid Growth Medium	1 Kit	05145
HepatiCult™ Organoid Growth Medium (Mouse)	100 mL	06030
Mouse Hepatic Organoids	2 Culture Wells	70932
PancreaCult™ Organoid Growth Medium (Mouse)	100 mL	06040
Mouse Pancreatic Organoids	200 Organoids	70933
STEMdiff™ Kidney Organoid Kit	1 Kit	05160
PneumaCult™ Airway Organoid Kit	1 Kit	05060
STEMdiff™ Cerebral Organoid Kit	1 Kit	08570
STEMdiff™ Cerebral Organoid Maturation Kit	1 Kit	08571
mTeSR™1	500 mL	85850
mTeSR™ Plus	1 Kit	05825



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