INTESTINAL ORGANOIDS

IntestiCult™ and STEMdiff™ Media for Intestinal Organoid Culture

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TABLE OF CONTENTS

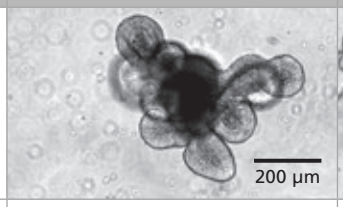
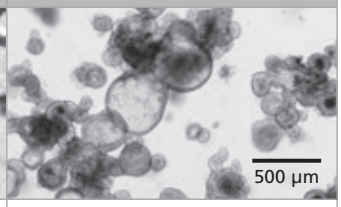
Introduction

The development of intestinal organoid culture techniques in 2009[1] has proven to be transformative for the landscape of intestinal and epithelial cell culture models across a wide variety of research interests and applications. Intestinal organoids are three-dimensional in vitro tissue models that self-assemble from human pluripotent stem cells (PSCs) directed to the intestinal lineage[2] or from intestinal adult stem cells (ASCs) cultured under the appropriate conditions.[3] To culture intestinal organoids, cells are embedded in an extracellular matrix and grown in a medium that mimics the signaling environment of the intestinal stem cell niche. These conditions provide the physical and chemical signaling sufficient for the epithelial cells to organize and polarize in a manner similar to what is observed in vivo. The organoids incorporate key intestinal cell types, including enterocytes, goblet cells, enteroendocrine cells and intestinal stem cells, recapitulating the cellular complement of the intestinal epithelium while removing confounding factors inherent to in vivo experimentation.

The presence of an actively dividing stem cell population enables expansion and maintenance of intestinal organoids in long-term culture. By providing a high degree of physiological relevance, easy maintenance and expansion as well as amenability to a wide range of in vitro experimental techniques, intestinal organoids offer significant experimental advantages and can complement or replace more traditional model systems for many applications.

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 3-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	• Mouse intestinal or colonic crypts • Mouse Intestinal Organoids (Catalog #70931)	• Human intestinal or colonic crypts • Established ASC-derived intestinal organoids	• 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	• Established common healthy and disease model strains • Tools for in vivo genetic manipulation	• Donor-specific genetic background • Targeted in vitro gene editing possible	• 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)	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. This medium supports efficient establishment, expansion and long-term maintenance of mouse intestinal epithelial organoids.

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. Has a convenient format and easy-to-follow protocol.

SERUM-FREE. Optimized formulation for low experimental variability.

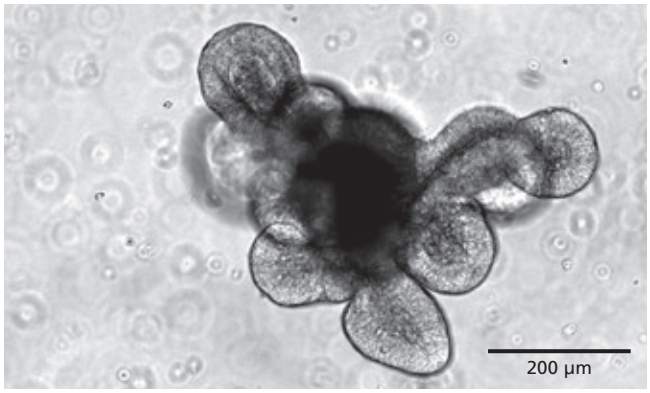


Figure 1. Light Microscope Visualization of a Mouse Intestinal Organoid

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

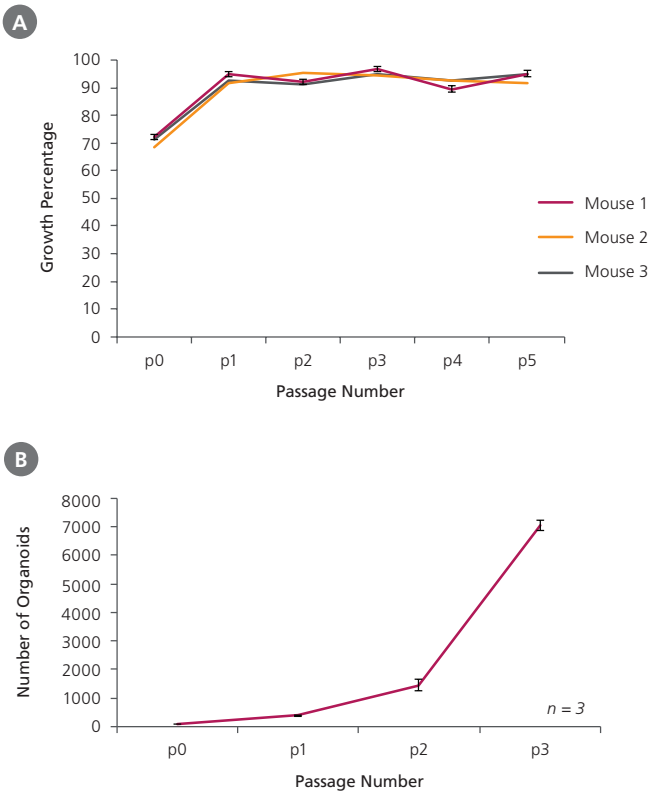


Figure 2. IntestiCult™ Organoid Growth Medium (Mouse) Supports Efficient Organoid Establishment and Expansion

(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. Organoids can be observed in culture in less than 1 week and can be maintained and expanded through passaging every 7 - 10 days.

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.

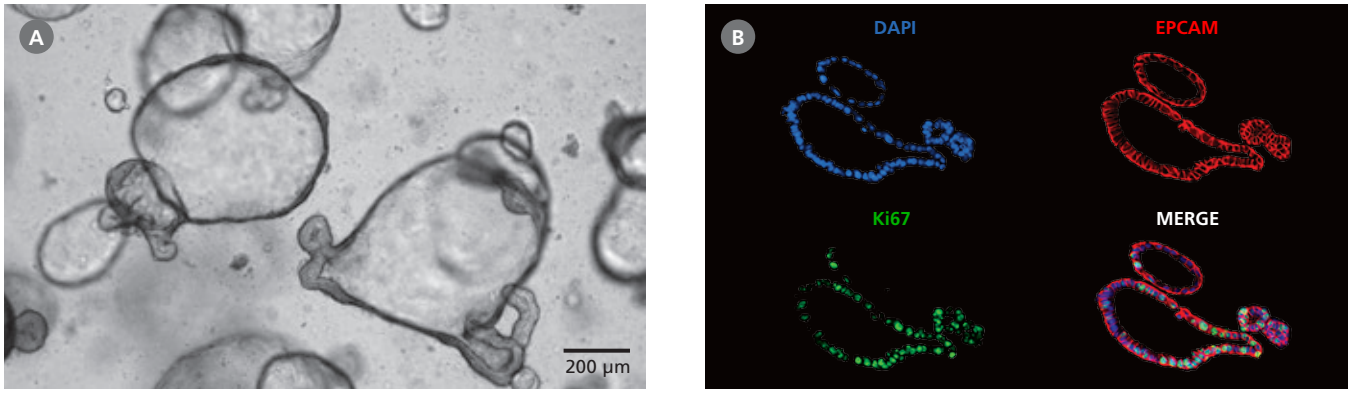


Figure 3. Human Colonic Organoids Grown in IntestiCult™ Organoid Growth Medium (Human)

(A) Colonic organoids grown in IntestiCult™ Organoid Growth Medium (Human) and imaged on day 7 of passage 0. (B) Immunofluorescence of intestinal organoids shows markers of mature epithelial cells (EPCAM, red) as well as of actively dividing intestinal progenitors (Ki67, green).

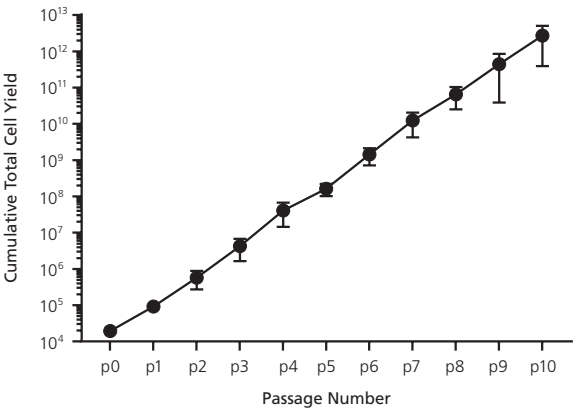


Figure 4. Intestinal Organoids can be Maintained in Long-Term Culture Through Passaging

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.

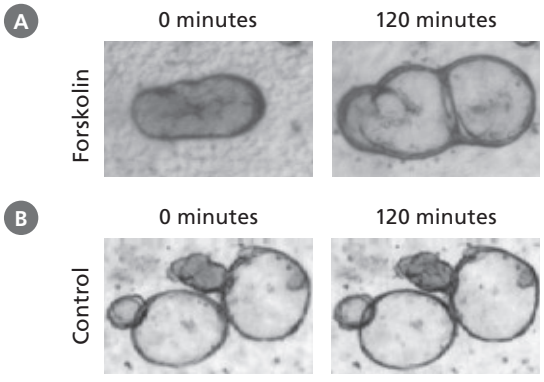


Figure 5. Forskolin-Induced Swelling of Intestinal Organoids

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.

STEMdiff™ Intestinal Organoid Kit

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

The STEMdiff™ Intestinal Organoid Kit supports efficient establishment of small intestinal organoid cultures from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) within 30 days. This serum-free medium kit is based on the formulation published by Spence et al.[2] and has been optimized to increase efficiency and reproducibility of organoid formation and expansion. STEMdiff™ Intestinal Organoid Growth Medium can be used to passage and maintain organoids long-term.

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 ESC and iPSC 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.

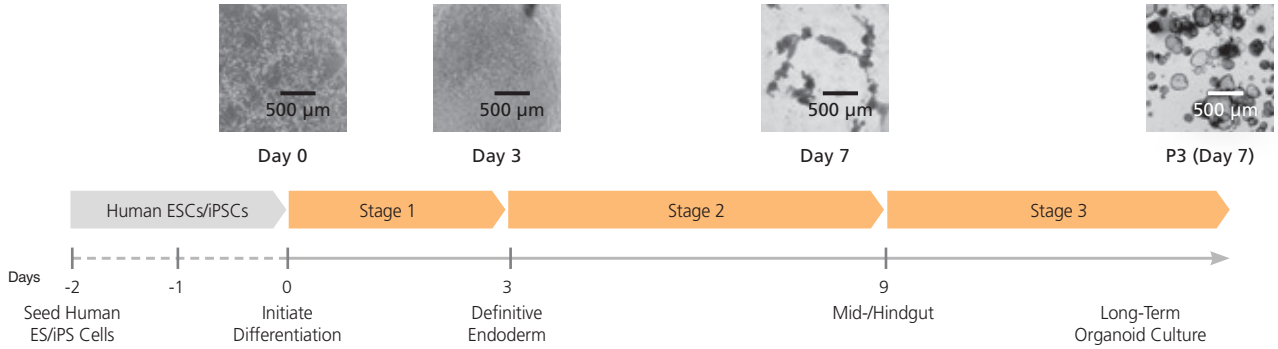


Figure 6. STEMdiff™ Intestinal Organoid Kit Enables Differentiation from hPSCs to Human Intestinal Organoids

hPSC 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 - 7), cells form mid-/hindgut spheroids that are released from the cell monolayer into the culture medium. These spheroids are collected, embedded in extracellular matrix, and cultured in STEMdiff™ Intestinal Organoid Growth Medium to mature into intestinal organoids.

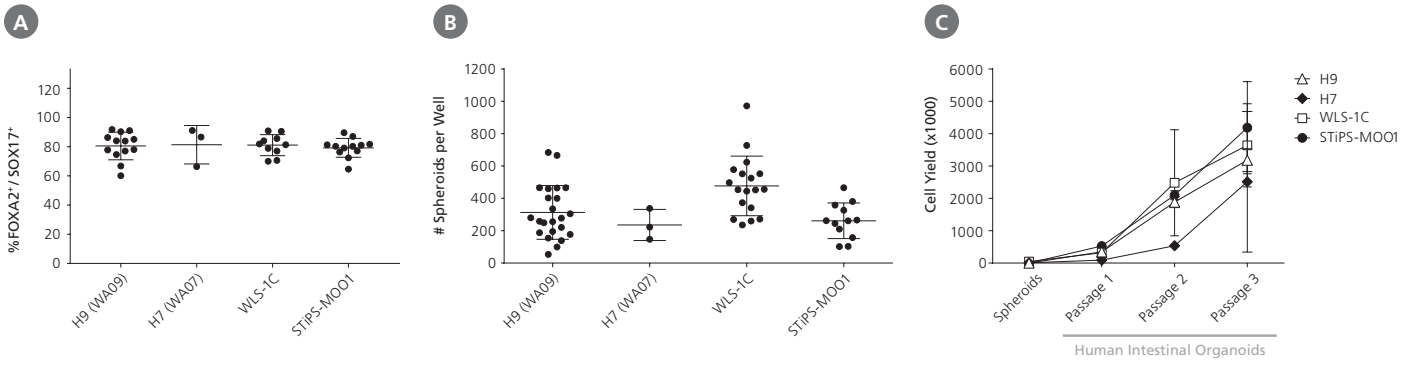


Figure 7. STEMdiff™ Intestinal Organoid Kit Supports Robust Differentiation and Expansion Across ESC and iPSC Lines

STEMdiff™ Intestinal Organoid Kit enables high-efficiency generation and expansion of intestinal organoids from both ESCs (H9, H7) and iPSCs (WLS-1C, STiPS-MOO1). (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 ESC- and iPSC-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 ESCs or iPSCs 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.

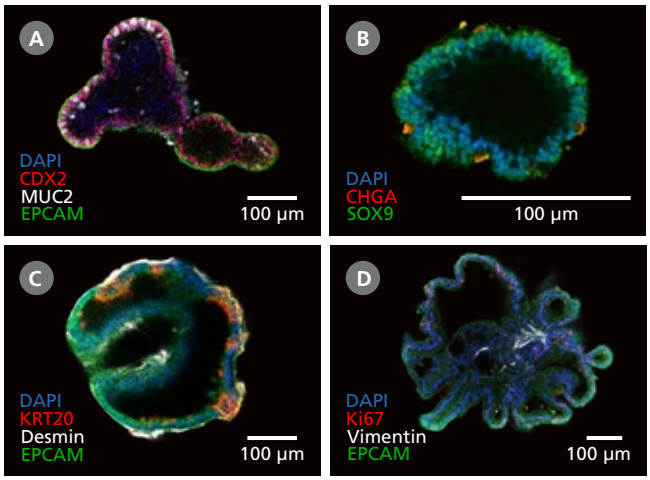


Figure 8. Characteristics of Intestinal Organoids Cultured Using STEMdiff™ Intestinal Organoid Growth Medium

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 are composed of 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 (A: goblet cells) and CHGA (B: enteroendocrine cells). (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 putative intestinal stem cells, respectively. Images are digital cross-sections of whole-mount immunofluorescence-stained intestinal organoids at P28 (Day 7).

Product Information

PRODUCT	QUANTITY	CATALOG #
IntestiCult™ Organoid Growth Medium (Mouse)	100 mL	06005
IntestiCult™ Organoid Growth Medium (Human)	100 mL	06010
STEMdiff™ Intestinal Organoid Kit	1 Kit	05140
STEMdiff™ Intestinal Organoid Growth Medium	100 mL	05145
Mouse Intestinal Organoids	200 Organoids	70931
mTeSR™1	500 mL	85850
CryoStor® CS10	100 mL	07930

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Modeling Human Gastrointestinal Development and Disease Using Pluripotent Stem Cells
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References

1. Sato T et al. (2009) Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 459(7244): 262–5.
2. Spence JR et al. (2011) Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. Nature. 470(7332): 105–9.
3. Sato T et al. (2011) Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 141(5): 1762–72.

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