CULTURING PSC-DERIVED HUMAN INTESTINAL ORGANOIDS

STEMdiff™ Intestinal Organoid Kit

Intestinal organoid culture techniques provide a unique platform for studying the intestine in vitro. Human pluripotent stem cell (PSC)-derived small intestinal organoids are three-dimensional cell cultures composed of a polarized intestinal epithelial monolayer. The apical cell surface surrounds the luminal compartment and the basolateral cell surface makes contact with the extracellular matrix and associated intestinal niche factor-producing mesenchyme. These organoids incorporate the main cell types and key functional features of the intestinal epithelium, providing direct relevance to the in vivo tissue. The organoids also maintain an actively dividing stem and progenitor cell population, allowing for expansion and maintenance of the cultures through long-term passaging, or cryopreservation for future experiments.

The increased complexity of organoids compared with cell line monolayers and the absence of confounding factors present in whole-animal models make organoids a convenient, flexible and relevant intestinal tissue model. As organoids exhibit donor-specific genotype and associated phenotype, they are useful as genetically diverse healthy and diseased tissue models. PSC-derived small intestinal organoids exhibit a fetal-like phenotype and are especially well-suited for studying the developing intestine.

Intestinal organoids can be generated from adult epithelial stem cells[1] or by directing differentiation of PSCs through the stages of intestinal development,[2] including formation of definitive endoderm (DE), mid-/hindgut specification and generation of intestinal epithelium. Early stages of the protocol are carried out in monolayer culture. During mid-/hindgut specification, cells self-organize into three-dimensional spheroids that are released from the monolayer into the culture medium; spheroids are collected, embedded in an extracellular matrix and exposed to a supportive culture medium to differentiate further and form intestinal organoids.

STEMdiff™ Intestinal Organoid Kit supports efficient establishment of PSC-derived small intestinal organoid cultures from induced pluripotent or embryonic stem cells (iPSCs or ESCs) 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.

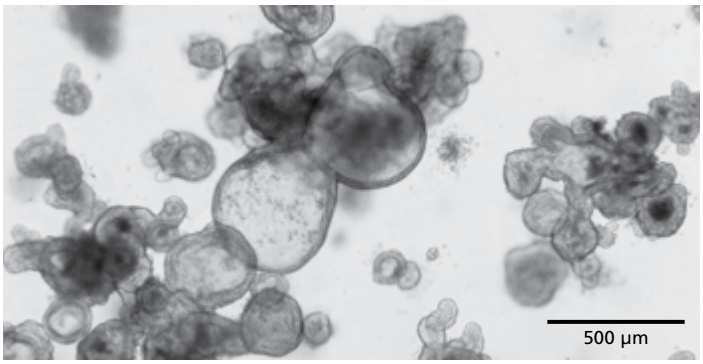

Figure 1. STEMdiff™ Intestinal Organoid Kit Enables the Growth of Intestinal Organoids from PSCs.

STEMdiff™ Intestinal Organoid Kit facilitates directed differentiation of PSCs to form human small intestinal organoids. The organoids exhibit a polarized epithelial monolayer surrounding a hollow lumen and are associated with a mesenchymal cell population. Pictured are passage 3 human intestinal organoids generated using this kit.

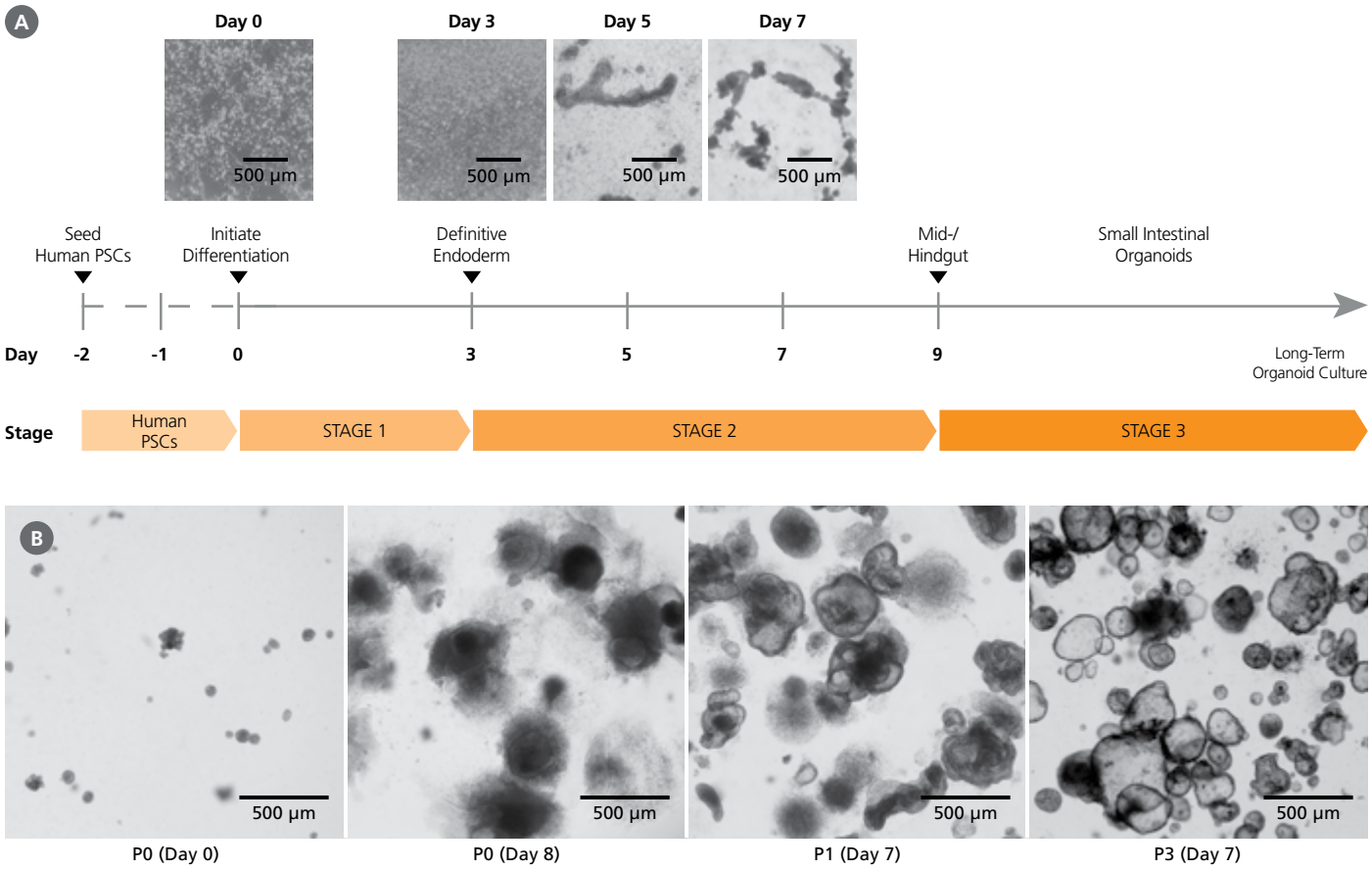

Figure 2. Generation of Human Intestinal Organoid Cultures Using the STEMdiff™ Intestinal Organoid Kit

(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.

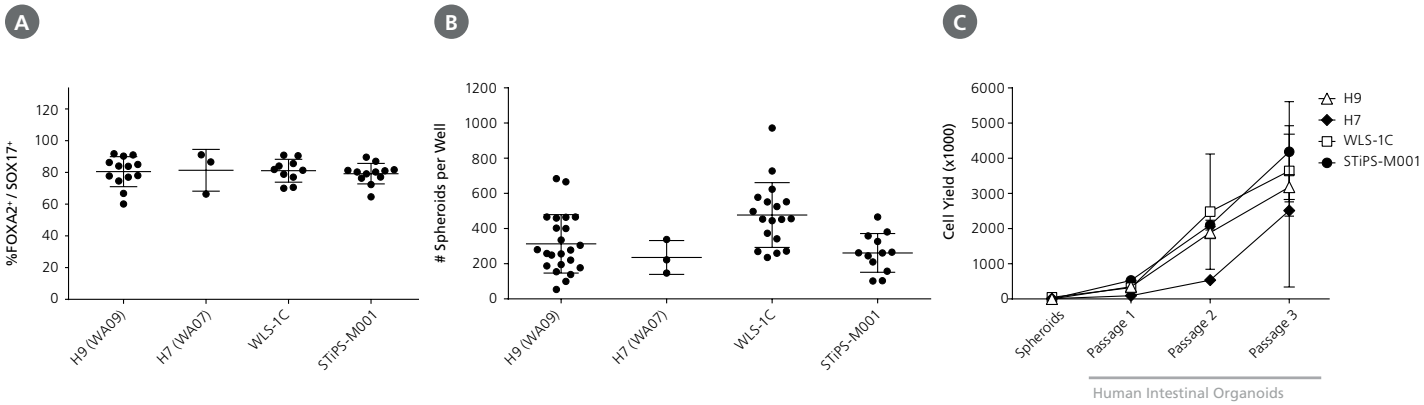

Figure 3. STEMdiff™ Intestinal Organoid Kit Supports Robust Differentiation and Expansion Across ESC and iPSC Lines

STEMdiff™ Intestinal Organoid Kit enables high-efficiency generation of intestinal organoids from both ESCs (H9, H7) and iPSCs (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 ESC- and PSC-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.

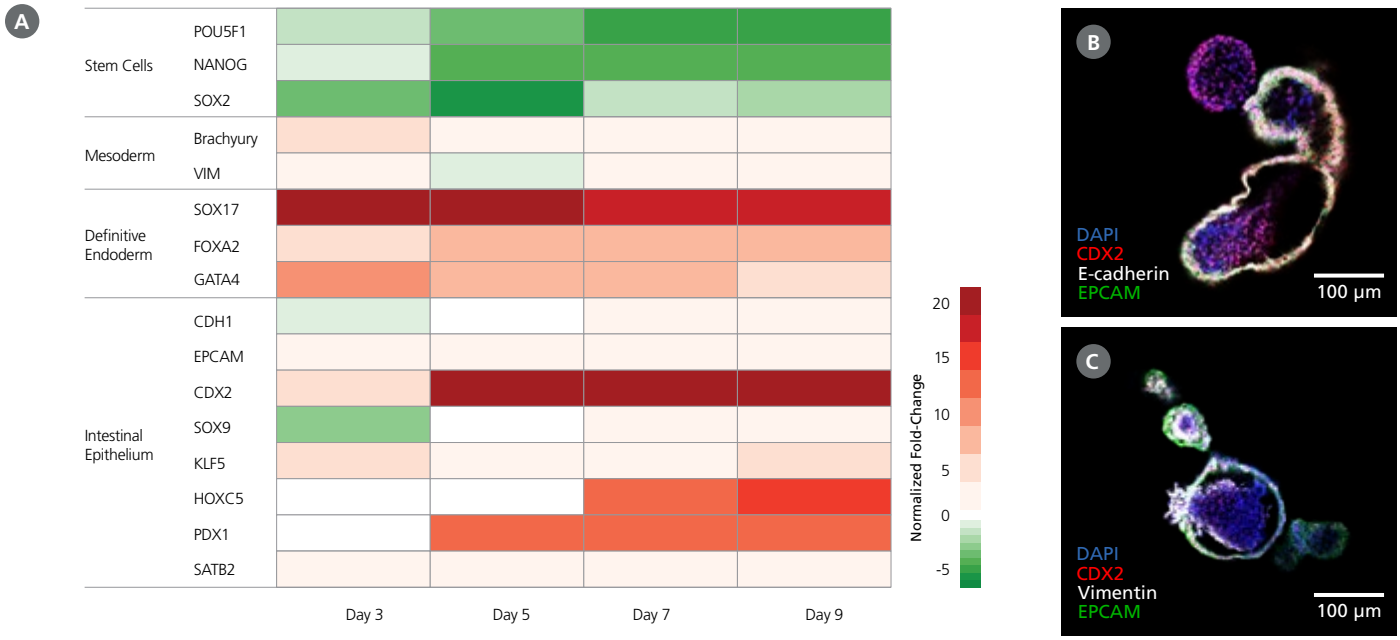

Figure 4. Characteristics of Mid-/Hindgut Spheroids Generated with STEMdiff™ Intestinal Organoid Kit

(A) Cultures differentiated using STEMdiff™ Intestinal Organoid Kit exhibit the expected markers during definitive endoderm and mid-/hindgut specification. During the protocol, gene expression patterns shift from pluripotency markers (day 0) to definitive endoderm markers by day 3 and those of the mid-/hindgut epithelium by day 9. Mid-/hindgut cultures (day 9) also express markers of the associated mesenchyme. Marker levels were assessed by RT-qPCR and normalized to expression levels for undifferentiatied H9 cells. (B) Mid-/hindgut spheroids (day 9) express markers of the intestinal epithelium (CDX2, E-cadherin, EPCAM). (C) Mid-/hindgut spheroids (day 9) also incorporate components of the associated mesenchyme (vimentin).

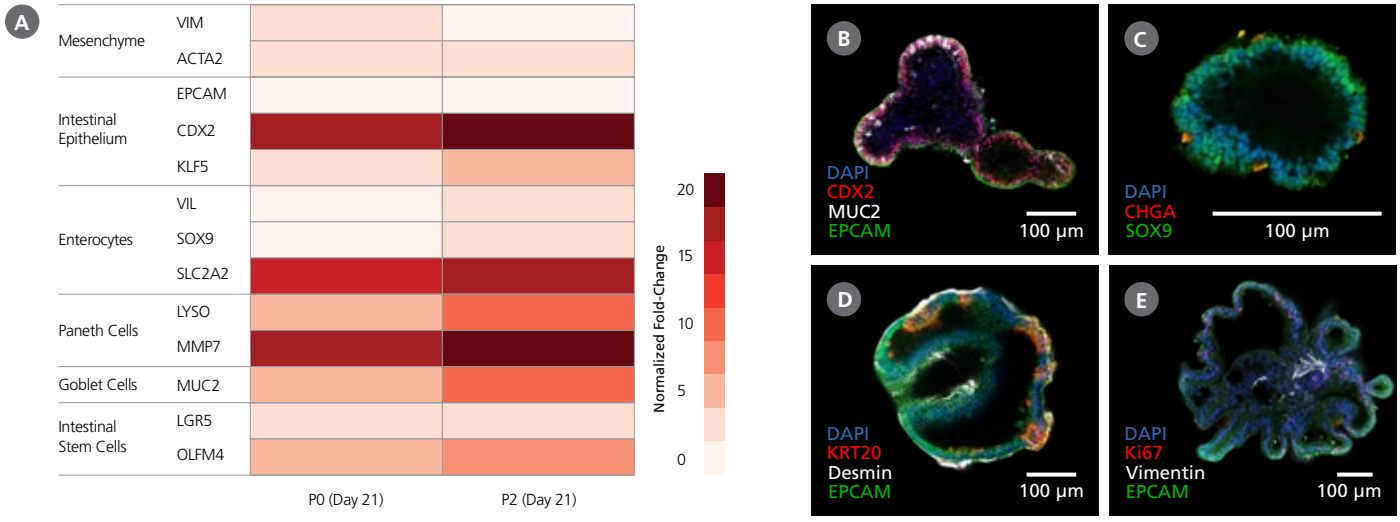

Figure 5. Intestinal Organoids Cultured In STEMdiff™ Intestinal Organoid Growth Medium Exhibit Features of the Intestinal Epithelium

(A) Differentiated PSC-derived intestinal organoids express markers of the intestinal epithelium and the associated mesenchyme. Marker levels were assessed by RT-qPCR and normalized to expression levels for undifferentiated H9 cells. (B,C) 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 (B), and express markers typical of mature cell types including MUC2 (B: goblet cells) and CHGA (C: enteroendocrine cells). (D,E) Observation of desmin (D) and vimentin (E) in intestinal organoids demonstrates incorporation of mesenchymal cells in the organoid cultures, while KRT20 (D) and Ki67 (E) 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	CATALOG #
STEMdiff™ Intestinal Organoid Kit	05140
STEMdiff™ Intestinal Organoid Growth Medium	05145
mTeSR™1	85850
CryoStor® CS10	07930

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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.