

# Forskolin-Induced Swelling of Human Intestinal Organoids Grown in IntestiCult™

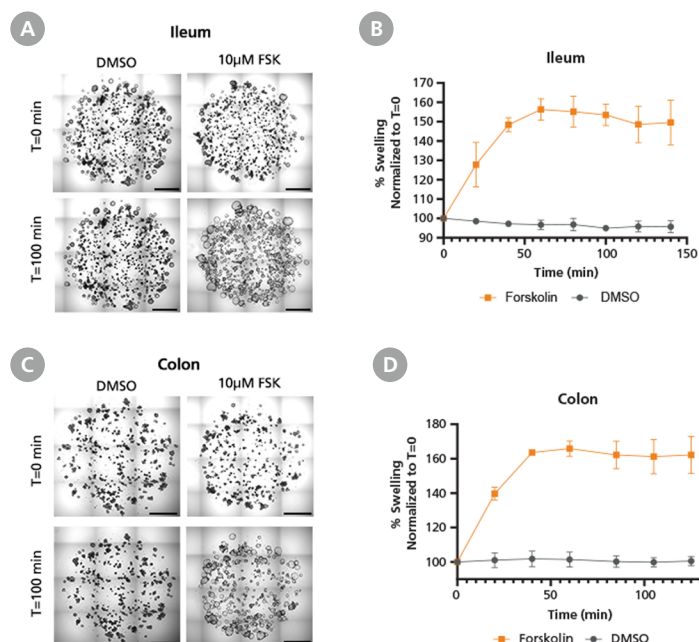
Below we describe a protocol for forskolin-induced swelling of human intestinal organoids derived from healthy individuals and cultured in IntestiCult™ Organoid Growth Medium (Human) (Catalog #06010) or IntestiCult™ Plus Organoid Growth Medium (Catalog #100-1677). For complete culturing instructions, use this document in conjunction with the relevant Product Information Sheet (Document [#DX21423](#) or [#10000031450](#)).

## Introduction

Cystic fibrosis (CF) is a genetic disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) that leads to defective chloride ion transport across epithelial tissues. These mutations result in the accumulation of thick mucus in multiple organs, including the lungs and intestine. The CFTR channel is therefore a key target for therapeutic development and CFTR-modulator drug discovery. Human intestinal organoids provide a physiologically relevant 3D cell culture system for studying CFTR function in vitro since they can be efficiently established from intestinal biopsies and expanded and maintained in long-term in vitro culture.<sup>1</sup> These 3D intestinal organoids maintain the genotype and phenotype of the parental tissue and retain the expected CFTR function when cultured in vitro, making them a powerful translational model for CFTR functional assays and phenotypic screening.

Exposing intestinal organoids to forskolin causes the cells to rapidly increase their levels of cyclic adenosine monophosphate (cAMP), resulting in the opening of the CFTR channel.<sup>1,2</sup> As chloride ions move through the channel and into the lumen, the organoids increase in size due to water following by osmosis. This organoid swelling can be quantified as a measurement of CFTR activity. Because the swelling is dependent on functional CFTR, wild type organoids swell normally, while those carrying a CFTR mutation or exposed to a CFTR inhibitor exhibit diminished or absent swelling in the presence of forskolin.

The forskolin-induced swelling (FIS) assay serves as a functional readout of CFTR channel activity in a native epithelial context, can be used to benchmark normal CFTR function, and serves as a reference for evaluating the effects of CFTR modulators, inhibitors, or other compounds targeting epithelial ion transport. By providing a reproducible, quantitative measure of CFTR-dependent fluid secretion in human-derived tissue, this organoid-based assay offers a robust platform for drug screening, mechanism-of-action studies, and preclinical evaluation of potential therapies for cystic fibrosis and related epithelial disorders.



**Figure 1. Organoids from Healthy Donors Cultured in IntestiCult™ Plus Are Responsive to Forskolin**

Representative brightfield images of healthy, tissue-derived intestinal organoids derived from the (A) ileum or (C) colon were expanded using IntestiCult™ Plus and exposed to 10 µM forskolin or a DMSO vehicle control for 100 minutes. (B) The quantified change in surface area of the healthy (B) ileal or (D) colonic organoids relative to T = 0 min following exposure to 10 µM forskolin or a DMSO vehicle control. Organoid swelling is evident in response to forskolin-induced activation of cystic fibrosis transmembrane conductance regulator (CFTR) activity.

## Materials

Product Name	Catalog #
IntestiCult™ Plus Organoid Growth Medium or IntestiCult™ Organoid Growth Medium	<a href="#">100-1677</a> or <a href="#">06010</a>
Forskolin	<a href="#">72112</a>
CFTR <sub>INH</sub> 172	<a href="#">100-0554</a>
GlyH-101	<a href="#">100-0530</a>
DMSO	-
24-Well Organoid Culture Plates or Costar® 24 Well Flat-Bottom Plate, Tissue Culture Treated	<a href="#">200-0561</a> or <a href="#">38017</a>
Krebs-Ringer Solution, Bicarbonate-Buffered	e.g. Alfa Aesar J67591

## Protocol

1. Establish organoid cultures as described in the Product Information Sheet (Document #DX21423 or #10000031450). Grow organoids for 3 - 5 days after passage in IntestiCult™ Organoid Growth Medium (Human) (Catalog #06010) or for 6 - 7 days after passage in IntestiCult™ Plus Organoid Growth Medium (100-1677) prior to assessing forskolin-induced swelling.
2. Remove media from each well and replace with an equal volume of Krebs-Ringer Bicarbonate (KBR) buffer. Allow the buffer to equilibrate with the Corning®Matrigel® domes for 30 minutes at 37°C.

**Note:** *Forskolin-induced swelling can be performed and assayed in the culture medium; however, swelling is more pronounced if using KBR buffer.*

3. OPTIONAL: If using CFTR inhibitors as an additional control (e.g. CFTR<sub>INH</sub>172 or GlyH-101), add the appropriate amount of inhibitor to each well as necessary (50 µM for CFTR<sub>INH</sub>172 or GlyH-101) and incubate for 3 hours at 37°C. If inhibitors are not being used, proceed directly to step 4.
4. Capture time = 0 images before proceeding as organoid swelling may begin immediately after adding forskolin.
5. Remove the buffer from all wells being assayed and replace with fresh buffer, inhibitors (if applicable), and forskolin to a final concentration of 10 µM forskolin.
6. Image each well at regular intervals until the swelling in uninhibited wells ceases (usually 80 - 120 minutes).
7. To quantify forskolin-induced swelling, brightfield images at each time point are analyzed using ImageJ for average organoid area compared to the time = 0 images.

## Related Products

Description	Catalog #
IntestiCult™ Plus Organoid Growth Medium (Human)	<a href="#">100-1677</a>
IntestiCult™ Organoid Growth Medium (Human)	<a href="#">06010</a>
Forskolin	<a href="#">72112</a>
CFTR <sub>INH</sub> 172	<a href="#">100-0554</a>
GlyH-101	<a href="#">100-0530</a>
DMSO	-
24-Well Organoid Culture Plates	<a href="#">200-0561</a>
Costar® 24-Well Flat-Bottom Plate, Tissue Culture Treated	<a href="#">38017</a>
Krebs-Ringer Solution, Bicarbonate-Buffered	e.g. Alfa Aesar J67591

## References

1. Dekkers, JF et. al (2013) A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat. Med.* 19(7): 939–47.
2. Boj, SF et. al (2017) Forskolin-induced swelling in intestinal organoids: An in vitro assay for assessing drug response in cystic fibrosis patients. *Journal of Visualized Experiments.* 120(e55159).

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