Strategies for optimizing the isolation and expansion of sensitive patient-derived duodenoids, ileoids and colonoids .pdf

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APR 28, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.kxygx9d6og8j/v1

Protocol Citation: Katlynn Bugda Gwilt, Jay R. Thiagarajah 2023. Strategies for optimizing the isolation and expansion of sensitive patient-derived duodenoids, ileoids and colonoids .pdf. **protocols.io** https://dx.doi.org/10.17504/protocols.io.kxygx9d6og8j/v1

Protocol status: Working
We use this protocol and it's working as described

Created: Apr 28, 2023

Last Modified: Apr 28, 2023

PROTOCOL integer ID: 81156

ABSTRACT

These protocols are an optimization of previous protocols and the original work described in Sato et al., 2009 which provided a foundation for the development of primary duodenoids, ileoids and colonoids from human samples. Here, we optimized methods specifically for use with samples from pediatric patients with genetic intestinal epithelial disorders. These protocols apply specifically to enteroids generated from endoscopic biopsies from patients with Congenital Diarrheas and Enteropathies (CoDE) or Very-Early-Onset Inflammatory Bowel Disease (VEO-IBD) or age-matched patients without any intestinal disease and include samples from the duodenum (duodenoids), ileum (ileoids), and colon (colonoids). The duodenoids, ileoids and colonoids from these patients exhibit a range of cellular phenotypes that potentially impact enteroid growth and maintenance including increased apoptosis, defects in proliferation, polarity and vesicular trafficking. These characteristics make it potentially challenging for long-term culture and expansion, limiting the availability of cells for functional experiments. Using a modified culture media, an expanded initial culture time after cell isolation from patient biopsies, and gentle passaging techniques, we were able to successfully culture and expand several patient lines over multiple passages and maintain the cultures for >5 years. The media conditions and protocols described here allow for reproducible phenotypes as well as scaling for larger functional studies on patient lines. These protocols also provide a useful starting point for further optimization for generating and culturing enteroids from patients with novel disease pathophysiology.

ATTACHMENTS

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BEFORE START

Dissolve all reagents to the appropriate concentration according to manufacturer's protocols. A * indicates that there were no modifications to the reagent.

MATERIALS

Key resources table		
Reagent	**Supplier**	**Catalog number**
TrypLE™ Express Enzyme (1X), phenol red	Thermo Fisher Scientific	Cat#12605010
Advanced DMEM/F12	Gibco	Cat#12634-028
L-WRN Conditioned Media	ATCC (Cite Clevers)	CRL-3276
Wnt Conditioned Media	ATCC	CRL-2647
R-spondin-1 conditioned Media	n/a	Hans Clevers
Noggin Conditioned Media	n/a	Hans Clevers
Recombinant Noggin	Peperotech	
Glutamax	Gibco	Cat#35050-061
HEPES	Gibco	Cat#15630-080
Primocin	Invivogen	Cat#Ant-pm-2
Normocin	Invivogen	Cat#Ant-nr-2
B27	Gibco	Cat#12587010
N2	Gibco	Cat#17502-048
Nicotinamide	Sigma-Aldrich	Cat#N0636
N-acetyl-cysteine	Sigma-Aldrich	Cat#A8199
A83-01	Sigma-Aldrich	Cat#SML0788
SB202190	Sigma-Aldrich	Cat#S7067
EGF	Peprotech	Cat#315-09
Gastrin	Sigma-Aldrich	Cat#G9145
Y27632	Sigma-Aldrich	Cat#Y0503
Prostaglandin E2	Sigma-Aldrich	Cat#P5640
CHIR99021	Sigma-Aldrich	Cat#SML1046
Corning® Matrigel® Growth Factor Reduced (GFR) Basement Membrane Matrix, Phenol Red-free, LDEV-free, 10 mL	Corning	Cat#356231
Cultrex	R&D Technologies	BME001-10
Cultrex Organoid Harvesting Solution, 100 mL	R&D Technologies	3700-100-01
Corning® Cell Recovery Solution, 100 mL	Corning	Cat#354253

Collagen IV from human placenta	Sigma-Aldrich	Cat#C5533
Collagenase, Type 1	Stem Cell	Catalog # 07416
6.5 mm Transwell with 0.4 µm pore polyester membrane insert, TC-treated, sterile, 48/cs	Corning	Cat#CLS3470-48EA
24 well plate	Costar	Cat#3526

BEFORE START INSTRUCTIONS

Dissolve all reagents to the appropriate concentration according to manufacturer's protocols. A * indicates that there were no modifications to the reagent.