

Generation, Maintenance, and Characterization of Human Pluripotent Stem Cell-derived Intestinal and Colonic Organoids

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Materials

Name	Company	Catalog Number	Comments
1% Bovine serum albumin (BSA) solution	N/A	N/A	N/A
15 mL Corning tube	Falcon	21008-918	N/A
30% Sucrose	N/A	N/A	Made in PBS.
5% Normal donkey serum	Jackson ImmunoResearch Lab	017-000-121	N/A
50 mL Corning tube	Falcon	21008-951	N/A
Accutase	Thermo Scientific	A1110501	Cell detachment solution; aliquot 5 mL of Accutase into 10 mL tubes totaling 20 tubes and store at -20 °C for up to 6 months. Place at 4 °C overnight before use.
Activin A	Cell guidance Systems	GFH6-100x10	Reconstitute the lyophilized powder at 100 µg/mL in sterile PBS containing 0.1% bovine serum albumin (BSA). Aliquot 38 µL of Activin A into prechilled microcentrifuge tubes and store at -80 °C (Tubes expire 12 months from date of receipt).
Activin Day 1 medium (RPMI 1640)	Corning	MT10041CV	Use nonessential amino acids (NEAA, Corning 11140050) and store at 4 °C. Basic day 1 medium: 500 mL of RPMI 1640 and 500 mL of NEAA. When preparing Activin Day 1 medium, add 13 mL of basic day 1 medium, 13 µL of Activin A (100 µg/mL), and 2 µL of BMP4 (100 µg/mL). The base medium is stable for up to 3 weeks but should be used immediately after addition of growth factors.
Activin Day 2 medium (RPMI 1640, 0.2% FBS vol/vol)	Hyclone	SH30070.03T	Use nonessential amino acids (Corning 11140050) and store at 4 °C. Basic day 2 medium: 500 mL of RPMI 1640, 500 mL of NEAA, and 1

			mL of 0.2% serum. When preparing Activin Day 2 medium, add 12.5 mL of basic day 2 medium and 12.5 μ L of Activin A (100 μ g/mL). The base medium is stable for up to 3 weeks but should be used immediately after addition of growth factors.
Activin Day 3 medium (RPMI 1640, 2% FBS vol/vol)	Hyclone	SH30070.03T	Use nonessential amino acids (Corning 11140050) and store at 4 °C. Basic day 3 medium: 500 mL of RPMI 1640, 500 mL NEAA, and 10 mL of 2% serum. When preparing Activin Day 3 medium, add 12.5 mL of basic day 3 and 12.5 μ L of Activin A (100 μ g/mL). The base medium is stable for up to 3 weeks but should be used immediately after addition of growth factors.
Alexa Fluor 488 Donkey anti-Goat	Thermo Scientific	A11055	1:500 dilution (Secondary antibody)
Alexa Fluor 488 Donkey anti-Rabbit	Thermo Scientific	A21206	1:500 dilution (Secondary antibody)
Alexa Fluor 546 Donkey anti-Mouse	Thermo Scientific	A10036	1:500 dilution (Secondary antibody)
Alexa Fluor 647 Donkey anti-Mouse	Thermo Scientific	A31571	1:500 dilution (Secondary antibody)
Base mold	Fisher	22-363-552	N/A
Basic gut medium (advanced DMEM)	Gibco	12491015	When preparing Basic gut medium, add 500 mL of DMEM, 500 mL of N2 (Gibco 17-502-048), 500 mL of B27 (Gibco), 500 mL of L-Glutamine to get 2 mM L-Glutamine (Corning A2916801), 5 mL of 100 U/mL Penicillin-Streptomycin (Gibco 15-140-122), and 7.5 mL of 1 M HEPES to get 15 mM HEPES. The base medium is stable for up to 3 weeks but should be used immediately after addition of growth factors.
Biorad CFX96 Touch Real-Time PCR Detection System	Biorad	N/A	Other qRT-PCR systems can be used.
Cell Recovery Solution	Corning	354253	ECM-degrading solution
CHIR99021	Reprocell	4000410	Reconstitute by adding 2.15 mL of DMSO at 10 mM. Prepare 50 μ L aliquots and store at -20 °C. Store powder at 4 °C, protected from light.
CTRL HIO patterning medium	N/A	N/A	Basic gut medium and 100 ng/mL EGF.
DAPI	Sigma-Aldrich	D9542	1:100 dilution (Secondary antibody)
DE monolayer	N/A	N/A	Monolayer was generated in prior steps (Section 4.4).
Dispase	Gibco	17105041	Resuspend lyophilized powder in Advanced DMEM (Gibco MT15090CV) to a 1 mg/mL final concentration. Filter the solution for sterilization by vacuuming using a Millipore filter sterilization tube. Make 10 mL aliquots (1 mg/mL) and store at -20 °C for up to 6 months. Place at 4 °C overnight before use.
EGF	Thermo Scientific	236-EG-01M	When preparing 100 ng/mL EGF reconstitute 500 μ g/mL in sterile PBS. Next add 2 mL of sterile PBS to 1 mg EGF and make 500 μ g/mL EGF solution. Aliquot 100 μ L of EGF in 20 tubes.

Fisherbrand 6 cm Petri Dishes with Clear Lid	Fisher	FB0875713A	N/A
Fisherbrand Cell Lifter	Fisher	08-100-240	N/A
Fisherbrand Class B Clear Glass Threaded Vials with Closures Attached	Fisher	03-338B	N/A
Fisherbrand Disposable Borosilicate Glass Pasteur Pipette	Fisher	13-678-2D0	N/A
Fluoromount G Slide Mounting Medium	VWR	100241-874	N/A
Gibco advanced DMEM	Gibco	12-491-023	N/A
Goat anti-E-Cadherin	R&D systems	AF648	1:400 dilution (Primary antibody)
Goat anti-SOX17	R&D systems	AF1924	1:500 dilution (Primary antibody)
HCOs patterning medium	N/A	N/A	Basic gut medium, 100 ng/mL EGF and 100 ng/mL BMP2. When preparing BMP2, add 1 mL of sterile 4 mM HCl 0.1% BSA to BMP2 vials (100 µg). Aliquot 25 µL of BMP4 solution in 4 tubes. The base medium is stable for up to 3 weeks but should be used immediately after addition of growth factors.
Hemocytometer	Sigma-Aldrich	Z359629	N/A
Human Pluripotent Stem Cells (hPSC)	Pluripotent Stem Cell Facility	N/A	Cells seeded in a Matrigel coated 24-well plate (Thermo Scientific 73520-906).
Ice-cold 4% Paraformaldehyde solution (PFA)	N/A	N/A	N/A
Ice-cold Phosphate Buffered Saline (PBS)	N/A	N/A	The pH must be 7.4.
ImmEdge Hydrophobic Barrier Pen	Vector Laboratories	101098-065	N/A
Induced Pluripotent Stem Cells (iPSCs)	Pluripotent Stem Cell Facility (Cincinnati Children's Hospital Medical Center)	N/A	Other hESC or iPSC lines can be used, but the protocol needs to be optimized for each cell line.
Leica microtome	N/A	N/A	N/A
LSM 880			confocal microscope
Matrigel Basement Membrane Matrix	Corning	354234	N/A
Matrigel hESC-qualified Matrix	Corning	354277	Prepare 4 x Matrigel aliquots which corresponds to volumes sufficient to make enough diluted Matrigel for 4 x 6-well dishes.
Mid-hindgut induction medium (RPMI 1640)	Corning	MT10041CV	Nonessential amino acids (Corning 11140050), 2% FBS vol/vol (Hyclone SH30070.03T), 3 µM CHIR99021 and 500 ng/mL FGF4. The base medium is stable for up to 3 weeks but should be used immediately after addition of growth factors.
Mid-hindgut spheroids	N/A	N/A	N/A
MilliporeSigma Steriflip Sterile Disposable Vacuum Filter Units	MilliporeSigma	SCGP00525	N/A
Mouse anti-CDX2	BioGenex	MU392-UC	1:300 dilution (Primary antibody)
Mouse anti-FOXA2	Abnova/Novus	H00003170-M01	1:500 dilution
mTeSR1 complete growth medium	Stem Cell technologies	85870	Add 100-mL of mTeSR supplement (85870) into one 400-mL mTeSR medium (85870) and aliquot into 50-mL tubes while avoiding contamination. Store at 4°C until use.

Murray's Clear solution (Also known as BABB)	Murray's	N/A	1:2 benzyl benzoate and benzyl alcohol.
NOG HIO patterning medium	N/A	N/A	Basic gut medium, 100 ng/mL EGF and 100 ng/mL NOGGIN (Dispense 25 µg of NOGGIN in 250 µl sterile PBS with 0.1% BSA).
NucleoSpin RNA	Takara	740955.25	Other RNA isolation kits may be used.
Nunclon delta surface tissue culture dish 24-wells (Nunc)	Thermo Scientific	73521-004	N/A
Nunclon delta surface tissue culture dish 24-wells coated with Matrigel	Thermo Scientific	73521-004	N/A
Nunclon delta surface tissue culture dish 6-wells (Nunc)	Thermo Scientific	73520-906	N/A
Nunclon delta surface tissue culture dish 6-wells coated with Matrigel.	Thermo Scientific	73520-906	N/A
Outgrowth medium for HIOs, CTRL HIOs, and HCOs	N/A	N/A	Basic gut medium and 100 ng/mL EGF (Final concentration)
Phosphate Buffer Saline, 0.5% Triton X (PBS-T)	N/A	N/A	N/A
Primers	Integrated DNA Technologies, Inc. (IDT)	N/A	The primers are listed in Table 2 on the protocol.
Rabbit anti-CDX2	Cell Marque	EPR22764Y	1:100 dilution (Primary antibody)
Rabbit anti-SATB2	Cell Marque	EP281	1:100 dilution (Primary antibody)
Recombinant Human BMP-4 Protein	R&D systems	314-BP-010	Reconstitute the lyophilized powder at 100 µg/mL in sterile 4 mM HCl containing 0.1% bovine serum albumin (BSA). Add 4.17 mL HCl solution to 45.83 mL molecular water totaling to 50 mL of 1 M HCl. Then add 200 µL of 1 M HCl to 49.8 mL of molecular grade water totaling to 50 mL of 4 mM HCl. Next add 0.05 g BSA to 50 mL of 4 mM HCl and filter to make sterile. Aliquot sterile 4 mM HCl 0.1% BSA to 33 microcentrifuge tube totaling and store at -20 °C. Add 100 µl of sterile 4 mM HCl 0.1% BSA to the BMP4 vials (10 µg) to make BMP4 solution at 100 µg/mL.
Recombinant Human FGF-4 Protein	R&D systems	235-F4-01M	Reconstitute at 100 µg/mL in sterile PBS containing 0.1% bovine serum albumin. Add 0.05 g of BSA in 50 mL of PBS to make 0.1% BSA. Filter 0.22 µM BSA to sterilize the BSA. Aliquot 10 mL of 0.1% BSA in 5 tubes. Add 1 mg FGF-4 in 10 mL of sterile 0.1% BSA. Aliquot 250 µL into prechilled 40 microcentrifuge tubes and store at -80 °C.
ROCK inhibitor Y-27632	Tocris	1254	The final concentration is 10 mM (10 mmol/L). Resuspend in DMSO at 10 mM and filter sterilize. Add 3 mL of sterile PBS to each vial. Aliquot 100 µL of ROCK inhibitor in 30 tubes and store at -20 °C.
SuperScript VILO cDNA Synthesis Kit	Thermo Scientific	11-754-250	N/A
SuperFrost Plus microscope slides			
Tissue Tek O.C.T Compound	VWR	25608-930	N/A