

# Advancements in Bovine Organoid Technology Using Small and Large Intestinal Monolayer Interfaces

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## Materials

Name	Company	Catalog Number	Comments
<b>Basal Medium</b>			
Advanced DMEM/F12 (1X)	Gibco	12634-010	n/a
GlutaMAX-I (100X)	Gibco	35050-061	2 mM
HEPES (1M)	Gibco	15630-080	10 mM
Pen Strep Glutamine (100X)	Gibco	10378-016	1X
<b>Organoid Culture Medium (Supplements to Basal Medium)</b>			
A-83-01	Sigma-Aldrich	SML0788-5MG	500 nM
B27 Supplement (50X)	Gibco	17504-001	1X
[Leu15]-Gastrin I human	Sigma-Aldrich	G9145-.5MG	10 nM
Murine EGF	PeproTech	315-09-500UG	50 ng/mL
Murine Wnt-3a	PeproTech	315-20-10UG	100 ng/mL
N-Acetyl-L-cysteine	MP Biomedicals	194603	1 mM
N-2 MAX Media Supplement (100X)	R&D Systems	AR009	1X
Nicotinamide	Sigma-Aldrich	N0636-100G	10 mM
Noggin Conditioned Medium	n/a	n/a	10 vol/vol %
Primocin	InvivoGen	ant-pm-2	100 µg/mL
R-Spondin-1 Conditioned Medium	n/a	n/a	20 vol/vol %
SB202190	Sigma-Aldrich	S7067-25MG	10 µM
<b>Monolayer Culture Medium (Supplements to Organoid Culture Medium)</b>			
CHIR99021	Sigma-Aldrich	SML1046-5MG	2.5 µM
HI FBS	Gibco	10438-034	20 vol/vol %
LY2157299	Sigma-Aldrich	SML2851-5MG	500 nM
Y-27632	StemCellTechnologies	72308	10 µM
<b>Reagents</b>			

Alexa Fluor 488 Mouse anti-E-cadherin	BD Biosciences	560061	1:200 dilution
Alexa Fluor 647 Phalloidin	Invitrogen	A22287	1:400 dilution
BSA	Cytiva	SH30574.02	2 w/vol %
Cell Recovery Solution	Corning	354253	n/a
DAPI Solution (1 mg/mL)	Thermo Scientific	62248	1:1000 dilution
DPBS (1X)	Gibco	14190-144	n/a
Fluorescein Isothiocyanate–Dextran	Sigma-Aldrich	FD4-100MG	0.5 mg/mL
Matrigel Matrix	Corning	354234	n/a
Paraformaldehyde Solution (4%)	Thermo Scientific	J19943K2	n/a
ProLong Gold antifade reagent	Invitrogen	P36930	n/a
SNA, EBL, Fluorescein	Vector Laboratories	FL-1301	1:100 dilution
Triton X-100	Thermo Scientific	A16046.AE	0.3 vol/vol %
TrypLE Express	Gibco	12605-028	n/a
Trypan Blue Solution, 0.4%	VWR Life Science	K940-100ML	n/a
<b>Materials and Equipment</b>			
0.4 µm Cell Culture Insert	Falcon	353095	
24-well Cell Culture Plate	Corning	3524	
48-well Cell Culture Plate	Thermo Scientific	150687	
70 µm Sterile Cell Strainer	Fisher Scientific	22-363-548	
96-well Cell Culture Plate	Greiner Bio-One	655086	
Centrifuge	Eppendorf	5910Ri	
CO <sub>2</sub> Incubator	Thermo Scientific	370	
Epithelial Volt-Ohm Meter	Millipore	Millicell ERS-2	
Hemocytometer	LW Scientific	CTL-HEMM-GLDR	
Inverted Confocal Microscope	Leica Microsystems	SP8-X	
Inverted Phase-Contrast Microscope	Leica Microsystems	DMI1	
Microscope Cover Glass	Fisher Scientific	12-540-B	
Microplate Reader	Molecular Devices	SpectraMax i3x	
Microscope Slides	Fisher Scientific	22-034-486	
Pasteur Pipets	Fisher Scientific	13-678-20C	
Scalpel Blade	iMed Scientific	-	#11 carbon steel
Vortex Mixer	Scientific Industries	SI-0236	
<b>Software</b>			
LAS X imaging software	Leica Microsystems	LAS X 3.7.6.25997	
Microplate Reader software	Molecular Devces	SoftMax Pro 7.1.2	