

Materials List for

Isolation and Enrichment of Human Lung Epithelial Progenitor Cells for Organoid Culture

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jove.com/video/61541

Materials

Name	Company	Catalog Number	Comments
Cell Isolation			
10 mL Sterile syringes, Luer-Lok Tip	Fisher scientific	BD 309646	
30 mL Sterile syringes, Luer-Lok Tip	VWR	BD302832	
Biohazard bags	VWR	89495-440	
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connecting ring	Pluriselect	41-50000-03	
Deoxyribonuclease (lot#SLBF7798V)	sigma Aldrich	DN25-1G	
Disposable Petri dishes	Corning/Falcon	25373-187	
Funnel	Pluriselect	42-50000	
HBSS	Corning	21-023	
Liberase TM Research Grade	sigma Aldrich	5401127001	
needle 16G	VWR	305198	
needle 18G	VWR	305199	
PluriStrainer 100 µm (Cell Strainer)	Pluriselect	43-50100-51	
PluriStrainer 300 µm (Cell Strainer)	Pluriselect	43-50300-03	
PluriStrainer 40 µm (Cell Strainer)	Pluriselect	43-50040-51	
PluriStrainer 500 µm (Cell Strainer)	Pluriselect	43-50500-03	
PluriStrainer 70 µm (Cell Strainer)	Pluriselect	43-50070-51	
Razor blades	VWR	55411-050	
Red Blood Cell lysis buffer	eBioscience	00-4333-57	
Equipment's			
GentleMACS C Tubes	MACS Miltenyi Biotec	130-096-334	
GentleMACS Octo Dissociator	MACS Miltenyi Biotec	130-095-937	
Leica ASP 300s Tissue processor			
LS Columns	MACS Miltenyi Biotec	130-042-401	
MACS MultiStand**	Miltenyi Biotec	130-042-303	
Thermomixer	Eppendorf	05-412-503	

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HBSS+ Buffer			
Amphotericin B	Thermo fisher scientific	15290018	2ml
EDTA (0.5 M), pH 8.0, RNase-free	Thermo fisher scientific	AM9260G	500µl
Fetal Bovine Serum	Gemini Bio-Products	100-106	10ml
HBSS Hank's Balanced Salt Solution 1X 500 ml	VWR	45000-456	500ml bottle
HEPES (1 M)	Thermo fisher scientific	15630080	5ml
Penicillin-Streptomycin-Neomycin (PSN) Antibiotic Mixture	Thermo fisher scientific	15640055	5ml
List of antibodies for FACS			
Alexa Fluor 647 anti-human CD326 (EpCAM) Antibody	BioLegend	369820	1:50
BD CompBead Anti-Mouse Ig, K/ Negative control particles set	Fisher Scientific	BDB552843	
CD31 MicroBead Kit, human	Miltenyi Biotec	130-091-935	20µl/ 10 ⁷ total cells
CD45 MicroBeads, human	Miltenyi Biotec	130-045-801	20µl/ 10 ⁷ total cells
DAPI	Sigma Aldrich	D9542-10MG	1:10000
FITC anti-human CD235a	BioLegend	349104	1:100
FITC anti-human CD31	BioLegend	303104	1:100
FITC anti-human CD45	BioLegend	304054	1:100
FITC anti-mouse IgM Antibody	BioLegend	406506	1:500
Mouse IgM anti human HT2-280	Terrace Biotech	TB-27AHT2-280	1:300
PE anti-human CD271(NGFR)	BioLegend	345106	1:50
Composition of Organoid Culture mediums			
MRC-5	ATCC	CCL-171	
PneumaCult -ALI Medium	Stemcell Technologies	5001	
Small Airway Epithelial Cell Growth Medium	PromoCell	C-21170	
ThinCert Tissue Culture Inserts, Sterile	Greiner Bio-One	662641	
Y-27632 (ROCK inhibitor) 100mM stock (1000x)	Stemcell Technologies	72302	
Mouse Basal medium:			
Amphotericin B	Thermo fisher scientific	15290018	50 µl
DMEM/F-12, HEPES	ThermoFisher scientific	11330032	50 ml
Fetal Bovine Serum	Gemini Bio-Products	100-106	5 ml
Insulin-Transferrin-Selenium (ITS -G) (100X)	ThermoFisher scientific	41400045	500 µl
Penicillin-Streptomycin-Neomycin (PSN) Antibiotic Mixture	Thermo fisher scientific	15640055	500 µl
SB431542 TGF-β pathway inhibitor (stock 100 mM)	Stem cell	72234	5 µl
List of antibodies for Immunohistochemistry			
Antigen unmasking solution, citric acid based	Vector	H-3300	937 µl in 100ml water
Histogel	Thermo Scientific	HG-4000-012	
Primary Antibodies			
Anti HT2-280	Terracebiotech	TB-27AHT2-280	1:500

FOXJ1 Monoclonal Antibody (2A5)	Thermo Fisher Scientific	14-9965-82	1:300
Human Uteroglobin/SCGB1A1 Antibody	R and D systems	MAB4218	1:300
Keratin 5 Polyclonal Chicken Antibody, Purified [Poly9059]	Biolegend	905901	1:500
MUC5AC Monoclonal Antibody (45M1)	Thermo Fisher Scientific	MA5-12178	1:300
PDPN / Podoplanin Antibody (clone 8.1.1)	LifeSpan Biosciences	LS-C143022-100	1:300
Purified Mouse Anti-E-Cadherin	BD biosciences	610182	1:1000
Sox-2 Antibody	Santa Cruz biotechnologies	sc-365964	1:300
Secondary Antibodies			
Donkey anti-rabbit IgG, 488	Thermo Fisher Scientific	A-21206	1:500
FITC anti-mouse IgM Antibody	BioLegend	406506	1:500
Goat anti-Hamster IgG (H+L), Alexa Fluor 594	Thermo Fisher Scientific	A-21113	1:500
Goat anti-Mouse IgG1 Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Thermo Fisher Scientific	A-21121	1:500
Goat anti-Mouse IgG2a Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Thermo Fisher Scientific	A-21131	1:500
Goat anti-Mouse IgG2a Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	Thermo Fisher Scientific	A-21134	1:500
Goat anti-Mouse IgG2b Cross-Adsorbed Secondary Antibody, Alexa Fluor 568	Thermo Fisher Scientific	A-21144	1:500
Buffers			
Immunohistochemistry Blocking Solution			3% BSA, 0.4% Triton-x100 in TBS (Tris based saline)
Immunohistochemistry Incubation Solution			3% BSA, 0.1% Triton-X100 in TBS
Immunohistochemistry Washing Solution			TBS with 0.1% Tween 20