

Materials List for

# Isolating Bronchial Epithelial Cells from Resected Lung Tissue for Biobanking and Establishing Well-Differentiated Air-Liquid Interface Cultures

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## Materials

Name	Company	Catalog Number	Comments
1,000 ohm test resistor	World Precision Instruments	N/A	Used to calibrate the EVOM2 Epithelial Voltohmmeter
4-[2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)ethynyl]-benzoic acid (EC 23)	Tocris	4011	Used in cBD medium
6-well Clear TC-treated Multiple Well Plates	Corning	3506	Used in the first step to grow the cells isolated from the bronchial ring
Airway Epithelial Cell Growth Medium Kit	PromoCell	C-21160	Used to compare to cBD medium
Bead Bath 20 Liter	Lab Armor	74220-720	Used to pre-warm cell culture solutions
BEGM Bronchial Epithelial Cell Growth Medium BulletKit	LONZA	CC-3170	Used to compare to cBD medium
Bovine albumin fraction V (BSA)	Thermo Fisher Scientific	15260037	Used in coating solution
Bovine pituitary extract (BPE)	Thermo Fisher Scientific	37000-015	Used in c-KSFM
Bronchial epithelial cell growth supplement (BEpiCGS)	ScienCell Research Laboratories	3262	Used in cBD medium
Bronchial epithelial cell medium-basal (BEpiCM-b)	ScienCell Research Laboratories	SCC3211-b	Used in cBD medium
Cell culture inserts; 12 mm Transwell with 0.4 µm pore polyester membrane insert	Corning	3460	Cell culture inserts used in the protocol
Cell culture inserts; 12-well inserts, 0.4 µm PET clear	CellQART made by SABEU	9310412	Cell culture inserts used to compare with Corning cell culture inserts
Cell culture inserts; 12-well ThinCert Tissue culture Inserts	Greiner Bio-One	82050-032	Cell culture inserts used to compare with Corning cell culture inserts
CELLSTAR flask, TC, PS, 250 ml, 75 cm <sup>2</sup>	Greiner Bio-One	658170	Used to expand the number cells
CFX Maestro 1.0	Bio-Rad	N/A	Software program for analyzing qPCR data generated with the CFX384 System

CFX384 Touch Real-Time PCR Detection System	Bio-Rad	1855484	qPCR detection system
Chopstick electrode set	World Precision Instruments	STX2	Used to measure electrical resistance in ALI-PBEC
CO <sub>2</sub> -Incubator	PHCbi	MCO-170AICUV-PE	Cell culture incubator used for mycoplasma free cell cultures
CO <sub>2</sub> -Incubator	Hereaus	Heracell 150	Cell culture incubator used for possibly mycoplasma infected cell cultures
Coolcell Container	Corning	432006	Used to cryopreserve cells at -80 °C before transfer to liquid N <sub>2</sub>
Countess 3 Automated cell counter	Thermo Fisher Scientific	AMQAX2000	Used to count cells and determine the cell concentration
Cryovials	Nalgene	479-3224	Used to cryopreserve cells in
D-Glucose	Avantor VWR BDH CHEMICALS	101174Y	Used in soft trypsin
Dimethyl sulfoxide (DMSO)	Avantor VWR	0231	Used in cell freeze medium
dNTP (10 mM)	Promega	U1515	Used in the synthesis of cDNA
Dulbecco's Modified Eagle's Medium (DMEM) + 4500 mg/l D-Glucose	STEMCELL Technologies	36250	Used in cBD medium
Dulbecco's Modified Eagle's Medium (DMEM) 4.5 g/l glucose with l-glutamine	LONZA	LOBE12-604F	Used in cBD medium to compare with DMEM from other manufacturers
Dulbecco's Modified Eagle's Medium (DMEM), high glucose, pyruvate	Thermo Fisher Scientific	41966029	Used in cBD medium to compare with DMEM from other manufacturers
Epidermal growth factor (EGF)	Thermo Fisher Scientific	37000-015	Used in c-KSFM
Ethylenediaminetetraacetic acid (EDTA)	Avantor VWR BDH CHEMICALS	443885J	Used in soft trypsin
EVOM2 Epithelial Voltohmmeter	World Precision Instruments	91799	Used with the chopstick electrode set to measure electrical resistance in ALI-PBEC
Fibronectin solution, Human	PromoCell	C-43060	Used in coating solution
Glutamax	Thermo Fisher Scientific	35050038	Used in cBD medium
Hanks balanced salt solution (HBSS)	ScienCell Research Laboratories	SCC0313	Used to dissolve protease XIV
IQ SYBR Green Super mix	Bio-Rad	170887	qPCR reagent
Isoproterenol hydrochloride, (-)-	Sigma-Aldrich	I-6504	Used in c-KSFM
Keratinocyte-SFM (KSFM)	Thermo Fisher Scientific	17005-034	Used in c-KSFM
Maxwell RSC Instrument	Promega	AS4500	Automated RNA isolation system
Maxwell RSC simplyRNA Tissue Kit	Promega	AS1340	Used to isolate total RNA with the Maxwell RSC Instrument
M-MLV Reverse transcriptase	Promega	M5301	Used in the synthesis of cDNA
M-MLV Reverse transcriptase 5X reaction buffer	Promega	M531A	Used in the synthesis of cDNA
MycoStrip	InvivoGen	rep-mys-10	Used to detect the presence of mycoplasma in cell culture samples
N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES)	Thermo Fisher Scientific	15630056	Used in cBD medium
Oligo(dT) <sub>15</sub>	Qiagen	79237	Used in the synthesis of cDNA
Penicillin/Streptomycin solution (Pen/Strep)	ScienCell Research Laboratories	SCC0513	Used as antibiotic in c-KSFM and cBD medium
Phosphate buffered saline (PBS)	LUMC pharmacy	N/A	Used in different steps of the protocol
Pneumacult-ALI Medium	STEMCELL Technologies	05002	Used to grow cells in the differentiation stage to compare to cBD medium

Pneumacult-Ex Plus Medium	STEMCELL Technologies	05040	Used to grow cells in the submerged stage to compare to cBD medium
Primer, ATP5B, forward	Integrated DNA Technologies	N/A	Nucleotide sequence: TCACCCAGGCTGGTTCAGA
Primer, ATP5B, reverse	Integrated DNA Technologies	N/A	Nucleotide sequence: AGTGGCCAGGGTAGGCTGAT
Primer, FOXJ1, forward	Integrated DNA Technologies	N/A	Nucleotide sequence: GGAGGGGACGTAAATCCCTA
Primer, FOXJ1, reverse	Integrated DNA Technologies	N/A	Nucleotide sequence: TTGGTCCCAGTAGTCCAGC
Primer, MUC5AC, forward	Integrated DNA Technologies	N/A	Nucleotide sequence: CCTTCGACGGACAGAGCTAC
Primer, MUC5AC, reverse	Integrated DNA Technologies	N/A	Nucleotide sequence: TCTCGGTGACAACACGAAAG
Primer, MUC5B, forward	Integrated DNA Technologies	N/A	Nucleotide sequence: GGGCTTTGACAAGAGAGT
Primer, MUC5B, reverse	Integrated DNA Technologies	N/A	Nucleotide sequence: AGGATGGTCGTGTTGATGCG
Primer, RPL13A, forward	Integrated DNA Technologies	N/A	Nucleotide sequence: AAGGTGGTGGTCGTACGCTGTG
Primer, RPL13A, reverse	Integrated DNA Technologies	N/A	Nucleotide sequence: CGGGAAGGGTTGGTGTTTCATCC
Primer, SCGB1A1, forward	Integrated DNA Technologies	N/A	Nucleotide sequence: ACATGAGGGAGGCAGGGGCTC
Primer, SCGB1A1, reverse	Integrated DNA Technologies	N/A	Nucleotide sequence: ACTCAAAGCATGGCAGCGGCA
Primer, TP63, forward	Integrated DNA Technologies	N/A	Nucleotide sequence: CCACCTGGACGTATTCCACTG
Primer, TP63, reverse	Integrated DNA Technologies	N/A	Nucleotide sequence: TCGAATCAAATGACTAGGAGGGG
Primocin	InvivoGen	ant-pm-2	Used as antimicrobial agent against bacteria, mycoplasma, and fungi in c-KSFM medium
Protease XIV	Sigma-Aldrich	P5147	Used for the enzymatic treatment of the bronchial ring
RNAasin Recombinant Ribonuclease inhibitor	Promega	N2515	Used in the synthesis of cDNA
Soybean trypsin inhibitor (SBTI)	Sigma-Aldrich	T9128	Used to inhibit the action of soft trypsin
T100 Thermal Cycler	Bio-Rad	1861096	Used in the synthesis of cDNA
TissueSAFE plus	MILESTONE MEDICAL	N/A	Vacuum transfer system for biological specimens
Trypan blue solution	Thermo Fisher Scientific	15250061	Used to count live- and dead cells
Trypsin 1:250	Thermo Fisher Scientific	27250-018	Used in soft trypsin
Type I collagen solution (PureCol)	Advanced BioMatrix	5005-B	Used in coating solution
Universal container, PP, with PE screw cap	Avantor VWR	216-2053	Used in the protocol for the Protease XIV treatment of the bronchial ring