Materials List for:

Culturing of Human Nasal Epithelial Cells at the Air Liquid Interface

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Materials

Name	Company	Catalog Number	Comments
Nasal speculum	Welch Allyn	Model 22009	9 mm, reusable polyproylene
Operating otoscope	Welch Allyn	Model 21700	
Rhino probe cell cuvette	Arlington Scientific	SY-96-092	bend the head of the cuvette a little more
12-well culture plates	Corning	3512	
Transwell membrane cell culture inserts	Corning	3460	
Tissue culture flask	Corning	430639 and 430641	T25 and T75 vented flask
RPMI 1640 media (with L-Glutamine)	Gibco	11875	
Bronchial/Tracheal Epithelial Cell Growth Medium	Cell applications	511-500	
BEGM Bronchial Epithelial Cell Growth Medium	Lonza	CC-3170	This comes as a kit of one bottle of medium and vials with supplements.
Sodium Bicarbonate 7.5% solution	Gibco	25080	Use as it is.
NuSerum	BD Biosciences	355104	Use as it is.
BAMBANKER freezing media	BAMBANKER	302-14681	Use as it is.
CoolCell	Biocision	HTBCS-136	(CryoPrep alcohol-free cell freezing container)
PureCol	AdvancedBioMatrix	5005-B	dilute 1:100 with sterile water (should be roughly 0.03 mg/ml)use 500 µl/well of a 12-well plate
HBSS with Ca^{2} and Mg^{2+}	Gibco	14025	
DNAse 1	Sigma	DN25	Dissolve at 1.5 mg/ml, which is a 100x solution, add 10 µl to each ml of media
Trypsin, 0.25%, 1x, with phenol red	Gibco	25200-056	Use at it is
Soy Bean Trypsin Inhibitor (SBTI)	Sigma	T6522	Use dissolved in HBSS at a concentration of 1 mg/ml
Retinoic acid	Sigma	R7632	Make it up 100 µM stock solution (see below), dilute to 10 µM with absolute alcohol, take 5 µl of this and add it to 1 ml BEGM media (our working solution). All-trans Retinol has a coefficient of extinction of 52,480 in ethanol at 325 nm. Dissolve 25 mg in 50 ml absolute ethanol. Serial dilutions need to be made, dilute 50 ml of

			stock in 450 ml absolute ethanol, take 50 ml of this, dilute in 450 ml absolute ethanol, take 50 ml of this, dilute in 450 ml absolute ethanol, and again take 50 ml of this, dilute in 450 ml absolute Ethanol, take 100 ml of this and dilute in 900 ml absolute ethanol and read in the spectrophotometer at 325 nm, this number is then multiplied by its' dilution factor, then divided by the extinction coefficient of 52,480, this will give a number in mM. Make a 100 mM stock (store at -20 °C) and from this stock a 10 mM aliquot is made, the daily retinoic acid needed would be 5 ml of this 10 mM aliquot in 995 ml ALI media. Example: 325nm reading is 1.15. 1.15 x 10,000 (dilution factor) / 52,480 = 0.219 = 219 mM, make a 100 mM stock from this, in absolute ethanol.
DMEM high glucose (1x), liquid, with L-glutamine and sodium pyruvate	Gibco	11995	
Bovine Pituitary Extract	Gibco	13028-014	Used for BEGM ALI media.
Nystatin	Sigma	N4014-50 mg	Make up a solution of 3.3 mg/ml.
Bovine Serum Albumin	Fisher Scientific	BP1600-100	Make up a solution of 10 mg/ml solution in milliQ water.
PneumaCult-ALI media	StemCell	5001	This comes as a kit of one bottle of medium and vials with supplements. For details see below.
Hydrocortisone	StemCell	7904	Dissolve 2.4 mg hydrocortisone in 1 ml ddH_2O (used to make the 200x Hydrocortisone stock solution)
Heparin Sodium Salt in PBS (2 mg/ml)	StemCell	7980	Use at it is.
Filter flasks (500 ml)	Corning	431097	0.22 µm pore size
Filter tubes (50 ml)	Millipore	SCGP00525	Steriflip, 0.22 µm pore size
Pipette tips - ART Barrier Tips	Molecular Bioproducts	2139RI, 2069RI, 2179RI	
Conical tubes, sterile, 15 ml and 50 ml			
ddH_2O, absolute ethanol, ice			
CO_2 incubator			
refrigerated centrifuge			
biological safety cabinet			
pipettes (p2, p200, p1000), 9 in glass pipettes			
gloves			
lab coat with tight cuffs			

Media and solutions used:	**Recipe**	**Used for**
General remark: always warm the media to room temperature		
BEGM media	• 500 ml BEGM Cell Application media with one aliquot of retinoic acid	(mix them half-half and filter)

	• 500 ml BEGM Lonza media with one aliquot of single quot supplements	• digestion of the biopsy
BEGM+ media	• 50 ml BEGM media	• maintaining cells on plastic in the plates (passage0)
	• 10 µl retinoic acid (working solution)	• seeding cells in the flask (passage1; partially)
		• maintain the cells in the flask
BEGM++ media	• 50 ml Lonza's BEGM media with supplements	• seeding fresh cells in 12-well plate on plastic
	• 1 ml Sodium BiCarb	• maintaining cells in the plates on plastic (passage0; only partially)
	• 2.5 ml NuSerum	• seeding cells in the flask (passage1; partially)
BEGM ALI media	• 250 ml DMEM-H	• Maintaining cells on the transwell before they go ALI
	• 250 ml BEGM including supplements	
	• 2 ml Bovine Pituitary Extract (12.5 mg/ml solution)	
	• 1 ml Nystatin (3.3 mg/ml solution)	
	• 75 µl BSA (10 mg/ml solution)	
200X Hydrocortisone Stock Solution (96 µl/ml solution)	• 200 µl dissolved hydrocortisone	• As a supplement to the PneumaCult-ALI Complete Base Medium for the last day in the flask and when cells are on inserts
	• 4250 µl dissolved sodium chloride	
	• 540 µl ddH_2O	
	• 10 µl absolute ethanol	
	Filter solution through 0.22 µm filter, aliquot solution and store at -20 °C, avoid additional freeze/thaw cycles	
PneumaCult-ALI Complete Base Media	• 450 ml PneumaCult-ALI Basal Medium	• Base medium for all three PneumaCult-ALI media
	• 50 ml PneumaCult 10x Supplement	• The PneumaCult media is light sensitive, thus always keep it in the dark
	(this is stable for 2 weeks at 2-8 °C or for up to 6 months at -20 °C)	
PneumaCult-ALI Maintenance Medium	Per 1 ml of PneumaCult-ALI Complete Base Medium add:	ALI culture on inserts
	• 10 µl PneumaCult 100x *Maintenance* Supplement	
	• 2 µl Heparin (of a 2 mg/ml stock solution)	

	• 5 µl Hydrocortisone (of a 96 µl/ml stock solution)	

