PneumaCult™-ALI Medium

Serum- and BPE-free medium for human airway epithelial cells cultured at the air-liquid interface or as airway organoids

Catalog #05001 1 Kit

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Product Description

PneumaCult™-ALI is a serum- and BPE-free medium for the culture of human airway epithelial cells at the air-liquid interface (ALI). Airway epithelial cells cultured in PneumaCult™-ALI Medium undergo extensive mucociliary differentiation to form a pseudostratified epithelium that exhibits morphological and functional characteristics similar to those of the human airway in vivo.

PneumaCult™-ALI Medium also supports the generation of differentiated airway organoids in a 3D culture system. For a detailed protocol, refer to the Technical Bulletin: A Sphere Culture Method for Mucociliary Differentiation of Primary Human Bronchial Epithelial Cells (Document #28216), available at www.stemcell.com or contact us to request a copy.

Together, PneumaCult™-ALI Medium and PneumaCult™-Ex Plus Medium (Catalog #05040) constitute a fully integrated BPE-free culture system for in vitro human airway modeling. This robust and defined system is a valuable tool for basic respiratory research, toxicity studies, and drug development.

Product Information

The following components are sold as part of the PneumaCult™-ALI Medium kit (Catalog #05001) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
PneumaCult™-ALI Basal Medium	05002	450 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
PneumaCult™-ALI 10X Supplement*	05003	50 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.
PneumaCult™-ALI Maintenance Supplement	05006	5 x 1 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.

*This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
D-PBS (Without Ca++ and Mg++)	37350
PneumaCult™-Ex Medium OR PneumaCult™-Ex Plus Medium	05008 OR 05040
HBSS, Modified (Without Ca++ and Mg++)	37250
Heparin Solution	07980
Hydrocortisone Stock Solution	07925/07926
12 mm Transwell® with 0.4µm Pore Polyester Membrane Insert, Sterile	Corning 3460
Animal Component-Free Cell Dissociation Kit • ACF Enzymatic Dissociation Solution • ACF Enzyme Inhibition Solution	05426
Trypan Blue	07050

Preparation of Reagents and Materials

Use sterile techniques when preparing the following. If preparing volumes other than the indicated examples, adjust accordingly.

PneumaCult™-ALI Complete Base Medium

The following example is for preparing 500 mL of base medium.

1. Thaw PneumaCult™-ALI 10X Supplement overnight at 2 - 8°C. Mix gently by inverting the vial; do not vortex.

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquoted supplement, use immediately. Do not re-freeze.
2. Add 50 mL PneumaCult™-ALI 10X Supplement to 450 mL PneumaCult™-ALI Basal Medium. Mix thoroughly.

   NOTE: If not used immediately, store PneumaCult™-ALI Complete Base Medium at 2 - 8°C for up to 2 weeks. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the individual components. After thawing the complete base medium, use immediately. Do not re-freeze.

PneumaCult™-ALI Maintenance Medium

NOTE: Only prepare enough PneumaCult™-ALI Maintenance Medium needed for section B of Directions for Use (Maintenance Phase).

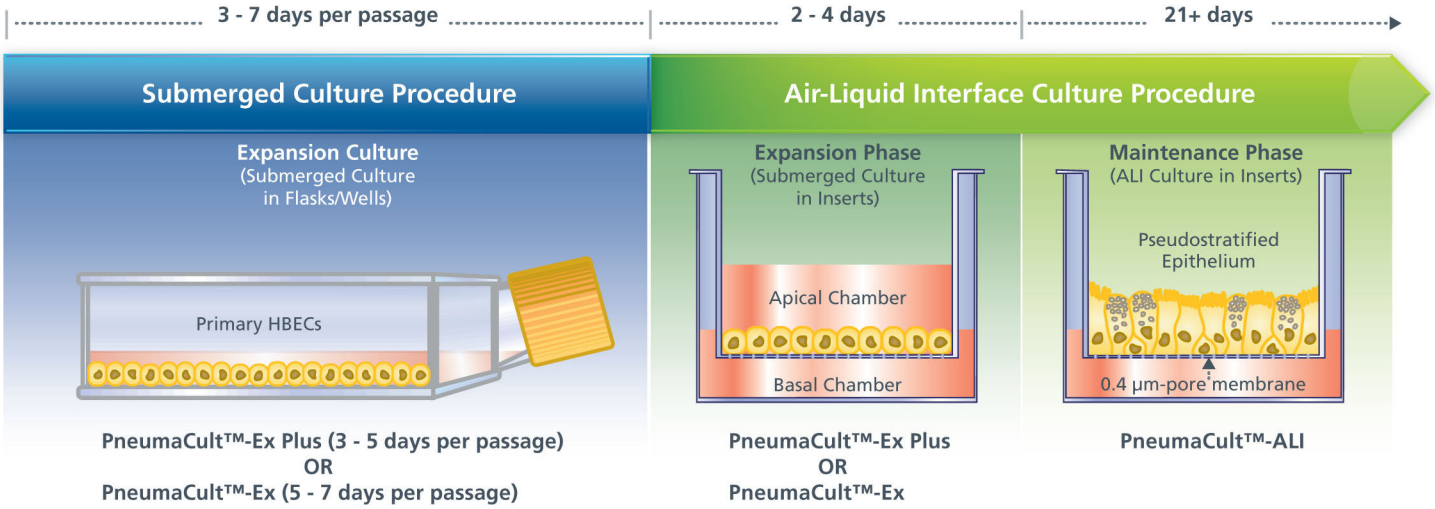
The following example is for preparing 10 mL of maintenance medium.

1. Thaw PneumaCult™-ALI Maintenance Supplement (100X) at room temperature (15 - 25°C).

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquoted supplement, use immediately. Do not re-freeze.
2. Combine the following components:
   - 9.83 mL PneumaCult™-ALI Complete Base Medium
   - 100 µL PneumaCult™-ALI Maintenance Supplement
   - 20 µL Heparin Solution
   - 50 µL Hydrocortisone Stock Solution

   NOTE: If not used immediately, store PneumaCult™-ALI Maintenance Medium at 2 - 8°C for up to 2 weeks.

Schematic of Culturing Human Airway Epithelial Cells



Directions for Use

Please read the entire protocol before proceeding.

A. EXPANSION PHASE (SUBMERGED CULTURE IN INSERTS)

The following example is for passaging human airway epithelial cells from a T-25 cm^2 flask and plating them on a single insert for a 12-well plate. If using other cultureware, adjust accordingly. PneumaCult™-Ex Medium may be substituted for PneumaCult™-Ex Plus Medium throughout the protocol.

NOTE: For complete instructions on expanding human airway epithelial cells in PneumaCult™-Ex Plus Medium or PneumaCult™-Ex Medium, refer to the Product Information Sheet (Document #DX21576 or 28201, respectively) available at www.stemcell.com or contact us to request a copy.

1. Warm sufficient volumes of D-PBS (Without Ca++ and Mg++), complete PneumaCult™-Ex Plus Medium, 0.025% Trypsin-EDTA, and 1 mg/mL Trypsin Inhibitor (soybean; in HBSS) to room temperature (15 - 25°C).
2. Wash cells with 5 mL D-PBS (Without Ca++ and Mg++).
3. Add 2 mL ACF Enzymatic Dissociation Solution and incubate at 37°C for 7 - 8 minutes, until cells can be dislodged with gentle tapping of the flask.
4. Add 2 mL ACF Enzyme Inhibition Solution and collect cells in a 15 mL conical tube (e.g. Catalog #38009).
5. Centrifuge the tube at 350 x *g* for 5 minutes.
6. Discard the supernatant and resuspend the cell pellet in 1 - 2 mL complete PneumaCult™-Ex Plus Medium.
7. Perform a viable cell count using Trypan Blue and a hemocytometer.
8. Plate 1 x 10^5 cells/cm^2 (e.g. 11 x 10^4 cells per Corning® 3460 insert) in 0.5 mL complete PneumaCult™-Ex Plus Medium in the apical chamber of the insert.
9. Incubate cells at 37°C and perform medium changes in both the basal (1 mL) and apical (0.5 mL) chambers every 2 days using PneumaCult™-Ex Plus Medium, until confluence is reached. This typically takes 2 - 4 days.

   NOTE: The expansion phase may take longer for some donor cell populations. Transitioning cultures that are < 50% confluent (PneumaCult™-Ex Plus Medium) or < 80% confluent (PneumaCult™-Ex Medium) is not recommended.
10. Continue to section B (Maintenance Phase).

B. MAINTENANCE PHASE (ALI CULTURE IN INSERTS)

1. Gently aspirate the medium from both the basal and apical chambers and add 1 mL of room temperature (15 - 25°C) PneumaCult™-ALI Maintenance Medium to the basal chamber only.
2. Incubate at 37°C and change medium in the basal chamber using PneumaCult™-ALI Maintenance Medium every 2 days, leaving the apical chamber empty.

   NOTE: On weekends, change the medium on Friday afternoon and first thing on Monday morning.
3. Beginning in week 2 post-airlift, remove excess mucus from the apical surface by washing the cells once with 0.5 mL of room temperature (15 - 25°C) D-PBS (Without Ca++ and Mg++). This procedure should be repeated as required (approximately once per week) to prevent excessive mucus accumulation.

   NOTE: Take care when removing liquid to avoid damaging the underlying cells.