PneumaCult™-ALI Medium

Serum- and BPE-Free Medium for Human Airway Epithelial Cells Cultured at the Air-Liquid Interface or as Sphere Cultures

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.

Together, PneumaCult™-ALI Medium and PneumaCult™-Ex Medium (Catalog #05008) 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.

PneumaCult™-ALI Medium also supports the generation of differentiated spherical structures of airway epithelial cells 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 on our website at www.stemcell.com or contact us to request a copy.

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	05008
Hanks' Balance Salt Solution (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
Trypsin-EDTA (0.05%)	07910
Trypsin Inhibitor from Glycine max (soybean)	Sigma-Aldrich T6522
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 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 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.

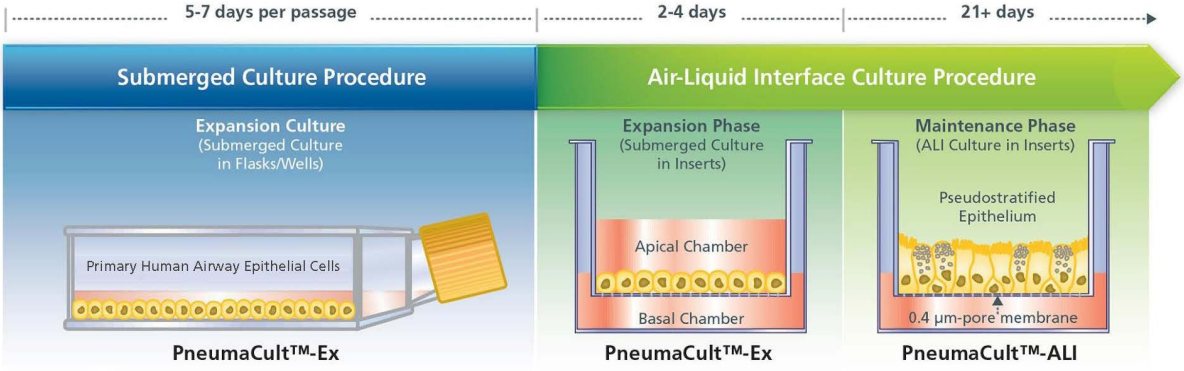
0.025% Trypsin-EDTA

Dilute 0.05% Trypsin-EDTA 1 in 2 in either Hanks' Balanced Salt Solution (HBSS) or phosphate-buffered saline (PBS).

1 mg/mL Trypsin Inhibitor

Prepare a 1 mg/mL solution of Trypsin Inhibitor (soybean) in HBSS.

Schematic of Human Airway Epithelial Cells Cultured in PneumaCult™-Ex Medium and PneumaCult™-ALI Medium



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.

NOTE: For complete instructions on expanding human airway epithelial cells in PneumaCult™-Ex Medium (Catalog #05008), please refer to the Product Information Sheet (Document #28201) available on our website 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 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 0.025% Trypsin-EDTA and incubate at 37°C for 3 - 5 minutes, until cells can be dislodged with gentle tapping of the flask.
4. Add 2 mL 1 mg/mL Trypsin Inhibitor (soybean; in HBSS) and collect cells in a 15 mL tube.
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 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 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 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 < 80% confluent 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 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 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.