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
Catalog #05021	1 Kit
Catalog #05022	1 Kit

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Product Description

PneumaCult™-ALI Medium (Catalog #05001) 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 is also available in a kit that includes 12 mm Transwell® inserts (Catalog #05021) or 6.5 mm Transwell® inserts (Catalog #05022).

Additionally, PneumaCult™-ALI Medium 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.

Ordering Information

PRODUCT NAME	CATALOG #	SIZE	KIT COMPONENTS
PneumaCult™-ALI Medium	05001	1 Kit	• PneumaCult™-ALI Basal Medium • PneumaCult™-ALI 10X Supplement • PneumaCult™-ALI Maintenance Supplement
PneumaCult™-ALI Medium with 12 mm Transwell® Inserts	05021	1 Kit	• PneumaCult™-ALI Medium (Catalog #05001) • Costar® 12 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts, 48 inserts (Catalog #38023)
PneumaCult™-ALI Medium with 6.5 mm Transwell® Inserts	05022	1 Kit	• PneumaCult™-ALI Medium (Catalog #05001) • Costar® 6.5 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts, 48 inserts (Catalog #38024)

Component Storage and Stability

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.
Costar® 12 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts	38023	48 Inserts	Store at 15 - 25°C.	Stable until expiry date (EXP) on label.
Costar® 6.5 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts	38024	48 Inserts	Store at 15 - 25°C.	Stable until expiry date (EXP) 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.
†This component is included in the PneumaCult™-ALI Medium kit (Catalog #05001) and is not available for individual sale.

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
Heparin Solution	07980
Hydrocortisone Stock Solution	07925
Costar® 12 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts* OR Costar® 6.5 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts**	38023* OR 38024**
Animal Component-Free Cell Dissociation Kit • ACF Enzymatic Dissociation Solution • ACF Enzyme Inhibition Solution	05426
Trypan Blue	07050

*Included in PneumaCult™-ALI Medium with 12 mm Transwell® Inserts kit (Catalog #05021)
**Included in PneumaCult™-ALI Medium with 6.5 mm Transwell® Inserts kit (Catalog #05022)

Preparation of Reagents and Materials

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

A. PneumaCult™-ALI Complete Base Medium

The following example is for preparing 500 mL of PneumaCult™-ALI Complete Base Medium (PneumaCult™-ALI 10X Supplement + PneumaCult™-ALI Basal Medium). Complete Base Medium is required for preparing Maintenance Medium (section B).

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 Complete Base Medium, use immediately. Do not re-freeze.

B. PneumaCult™-ALI Maintenance Medium

NOTE: Prepare only the volume of PneumaCult™-ALI Maintenance Medium required in section B of Directions for Use (Maintenance Phase).

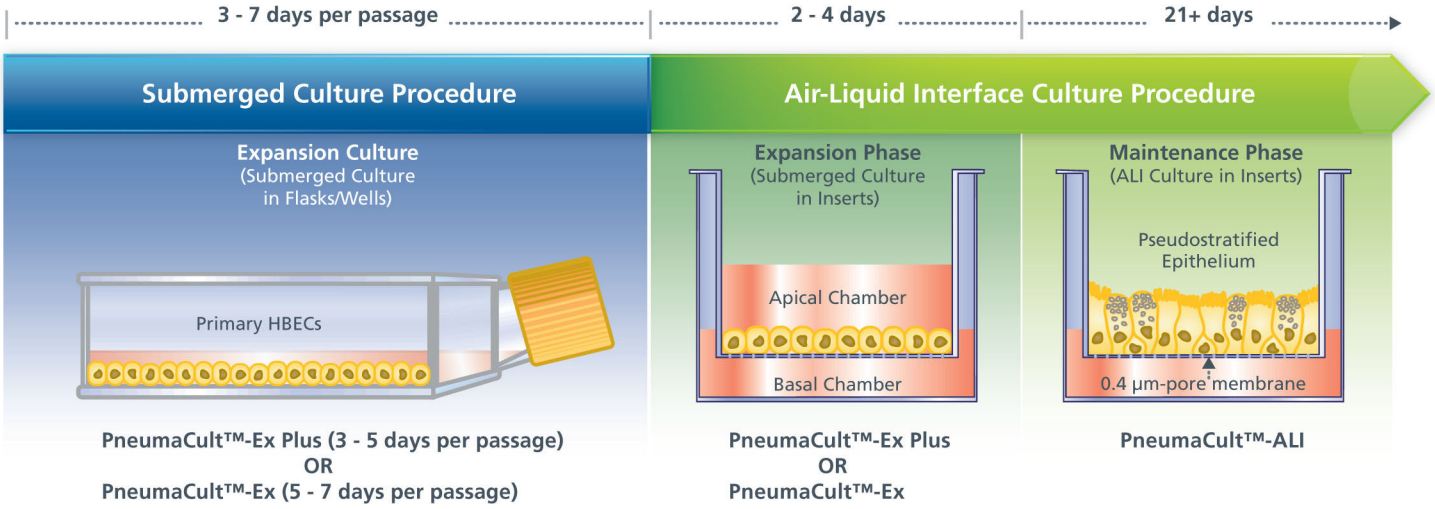
The following example is for preparing 10 mL of PneumaCult™-ALI Maintenance Medium (PneumaCult™-ALI Complete Base Medium + PneumaCult™-ALI Maintenance Supplement + Heparin Solution + Hydrocortisone Stock Solution).

1. Thaw PneumaCult™-ALI Maintenance Supplement 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 onto a single insert (in the 12-well or 24-well format). 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++), PneumaCult™-Ex Plus Medium, ACF Enzymatic Dissociation Solution, and ACF Inhibition Solution 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 PneumaCult™-Ex Plus Medium.
7. Perform a viable cell count using Trypan Blue and a hemocytometer.
8. Add PneumaCult™-Ex Plus Medium to one well of the tissue culture plate (basal chamber) as follows:
   - 12-well plate: 1 mL medium
   - 24-well plate: 0.5 mL medium
9. Plate 1 x 10^5 cells/cm^2 in the insert (apical chamber) as follows:
   - 12 mm Transwell® insert (12-well plate): 11 x 10^4 cells in 0.5 mL PneumaCult™-Ex Plus Medium
   - 6.5 mm Transwell® insert (24-well plate): 3.3 x 10^4 cells in 0.2 mL PneumaCult™-Ex Plus Medium
10. Incubate at 37°C. Perform full medium changes in both the basal and apical 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.
11. 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. Add PneumaCult™-ALI Maintenance Medium to the basal chamber only, as follows:
   - 12-well plate: 1 mL medium
   - 24-well plate: 0.5 mL medium
2. Incubate at 37°C. Perform a full medium change 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 Monday morning.
3. Beginning in week 2 post-airlift, remove excess mucus from the apical surface by washing the cells once with D-PBS (Without Ca++ and Mg++) at room temperature (15 - 25°C) as follows:
   - 12-well plate: 0.5 mL D-PBS
   - 24-well plate: 0.2 mL D-PBS

   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.