

# PneumaCult™-ALI-S Medium

**Serum- and BPE-free medium for human small airway epithelial cells cultured at the air-liquid interface**

Catalog #05050

1 Kit



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## Product Description

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

Together, PneumaCult™-ALI-S Medium and PneumaCult™-Ex Plus Medium (Catalog #05040) constitute a fully integrated serum- and BPE-free culture system for in vitro human small 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 a complete kit (Catalog #05050) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
PneumaCult™-ALI-S Basal Medium	05051	450 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
PneumaCult™-ALI-S Supplement (10X)*	05052	50 mL	Store at -20°C.	Stable for 2 years from date of manufacture (MFG) on label.
PneumaCult™-ALI-S Maintenance Supplement (100X)	05053	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 Plus Medium	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 <ul style="list-style-type: none"><li>• ACF Enzymatic Dissociation Solution</li><li>• ACF Enzyme Inhibition Solution</li></ul>	05426
Trypan Blue	07050

## Preparation of Reagents and Materials

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

### A. PneumaCult™-ALI-S Complete Base Medium

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

1. Thaw PneumaCult™-ALI-S Supplement (10X) 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-S Supplement (10X) to 450 mL PneumaCult™-ALI-S Basal Medium. Mix thoroughly.  
NOTE: If not used immediately, store PneumaCult™-ALI-S 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-S Maintenance Medium**

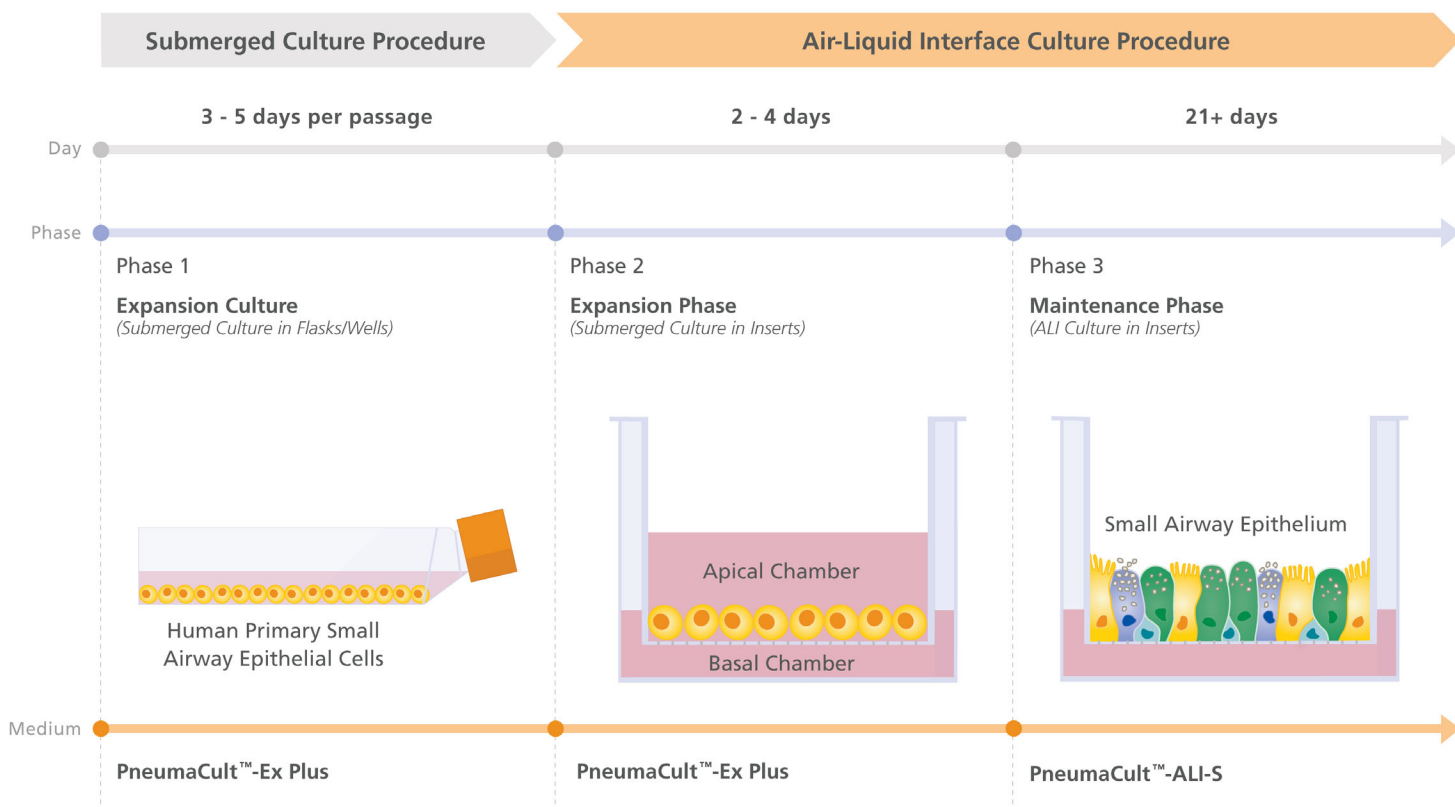
The following example is for preparing 10 mL of PneumaCult™-ALI-S Maintenance Medium (PneumaCult™-ALI-S Complete Base Medium + PneumaCult™-ALI-S Maintenance Supplement + Heparin Solution + Hydrocortisone Stock Solution).

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

1. Thaw PneumaCult™-ALI-S 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-S Complete Base Medium
  - 100 µL PneumaCult™-ALI-S Maintenance Supplement (100X)
  - 20 µL Heparin Solution
  - 50 µL Hydrocortisone Stock Solution

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

**Schematic of Culturing Human Small 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 small airway epithelial cells from a T-25 cm<sup>2</sup> flask and plating them onto a single Transwell® insert (in the 12-well or 24-well format). If using other cultureware, adjust accordingly.

NOTE: For complete instructions on expanding human airway epithelial cells in PneumaCult™-Ex Plus Medium, refer to the corresponding Product Information Sheet available at [www.stemcell.com](http://www.stemcell.com) or contact us to request a copy.

1. Warm sufficient volumes of D-PBS (Without Ca<sup>++</sup> and Mg<sup>++</sup>), 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<sup>++</sup> and Mg<sup>++</sup>).
3. Add 2.5 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 2.5 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<sup>5</sup> cells/cm<sup>2</sup> in the insert (apical chamber) as follows:
  - 12 mm Transwell® insert (12-well plate): 11 x 10<sup>4</sup> cells in 0.5 mL PneumaCult™-Ex Plus Medium
  - 6.5 mm Transwell® insert (24-well plate): 3.3 x 10<sup>4</sup> 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 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-S 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-S Maintenance Medium every 2 days, leaving the apical chamber empty.

NOTE: On weekends, change the medium on Friday afternoon and Monday morning.

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