PneumaCult™-ALI-S Medium

Serum- and bovine pituitary extract (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 bovine pituitary extract (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 2 years 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 2 years 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 #
Animal Component-Free Cell Dissociation Kit • ACF Enzymatic Dissociation Solution • ACF Enzyme Inhibition Solution	05426
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
D-PBS (Without Ca++ and Mg++)	37350
Heparin Solution	07980
Hydrocortisone Stock Solution	07925
PneumaCult™-Ex Plus Medium	05040
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).

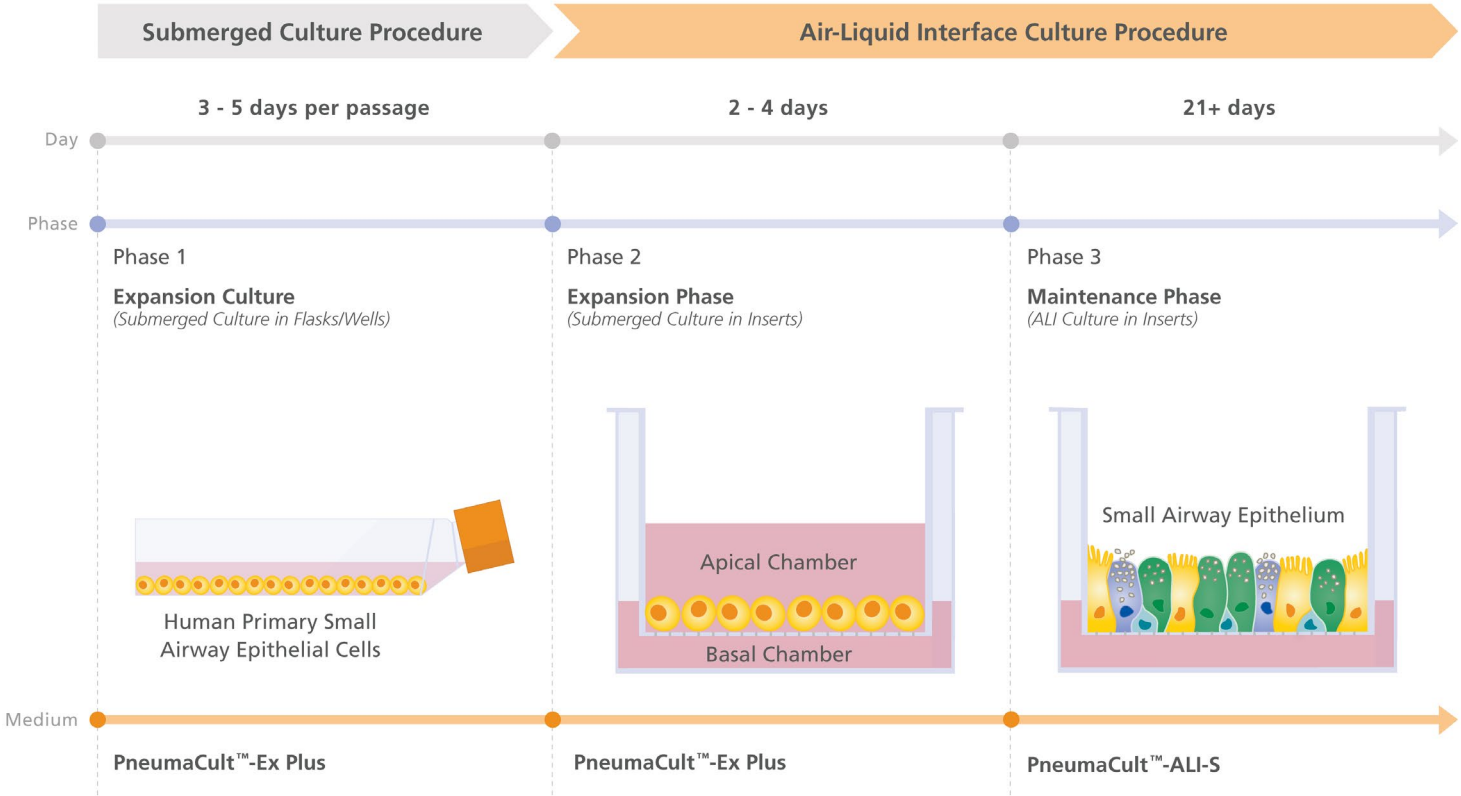
   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquoted supplement, use immediately. Do not re-freeze.

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^2 flask and plating them onto a single Transwell® insert (in the 12- 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, 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 2.5 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 (e.g. Catalog #100-1181).
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 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.

Related Products

For related products, including specialized cell culture and storage media, matrices, antibodies, cytokines, and small molecules, visit www.stemcell.com/hPSCworkflow, or contact us at techsupport@stemcell.com.