PneumaCult™-NGEx Medium

Serum- and BPE-free medium for expansion of primary human bronchial, small airway, and nasal epithelial cells

Catalog #100-1505 500 mL

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Product Description

PneumaCult™-NGEx Medium is an optimized serum- and BPE-free cell culture medium for the improved expansion of primary human airway epithelial cells, including bronchial epithelial cells (HBECs), small airway epithelial cells (HSAECs), and nasal epithelial cells (HNECs). PneumaCult™-NGEx Medium builds upon previous formulations of PneumaCult™ media (e.g. PneumaCult™-Ex Plus) to support at least 15 passages of expansion, generating approximately 250X more HBECs in three weeks compared to other commercially available expansion media. The resulting cells display better-conserved differentiation potential and function, defined as the ability to form a pseudostratified mucociliary epithelium at the air-liquid interface (ALI) using PneumaCult™-ALI Medium (Catalog #05001) or a cuboidal epithelium using PneumaCult™-ALI-S Medium (Catalog #05050).

Product Information

The following components are sold as a complete kit (Catalog #100-1505) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
PneumaCult™-NGEx Basal Medium	100-1504	490 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
PneumaCult™-NGEx 50X Supplement*	100-1503	10 mL	Store at -20°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.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
Hydrocortisone Stock Solution	07925
Animal Component-Free Cell Dissociation Kit • ACF Enzymatic Dissociation Solution • ACF Enzyme Inhibition Solution	05426
Conical tube, 15 mL	e.g. 38009
D-PBS (Without Ca++ and Mg++)	37350
Trypan Blue	07050
Tissue culture-treated T-25 cm^2 flask, with vented cap	e.g. Corning 353109

Preparation of Medium

Use sterile technique to prepare complete PneumaCult™-NGEx Medium (Basal Medium + 50X Supplement + Hydrocortisone Stock Solution). The following example is for preparing 500 mL of complete medium. If preparing other volumes, adjust accordingly.

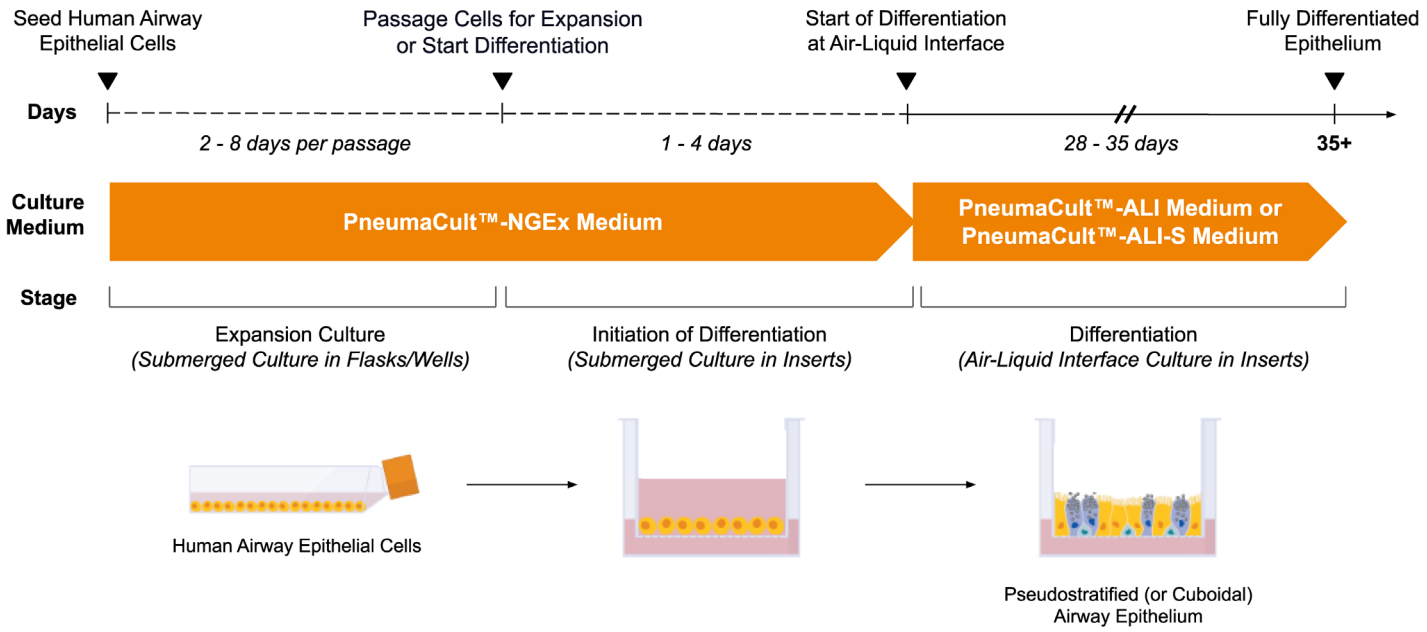
1. Thaw PneumaCult™-NGEx 50X Supplement at room temperature (15 - 25°C). Mix gently by inverting the vial; do not vortex.

   NOTE: A precipitate may be observed after thawing. This will not affect performance if the supplement is gently mixed.

   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 10 mL PneumaCult™-NGEx 50X Supplement and 0.5 mL Hydrocortisone Stock Solution to 490 mL PneumaCult™-NGEx Basal Medium. Mix thoroughly.

   NOTE: If not used immediately, store complete PneumaCult™-NGEx Medium at 2 - 8°C for up to 4 weeks. Do not exceed the shelf life of the individual components.
3. If desired, add antibiotics and antifungals immediately before use (e.g. 50 µg/mL gentamicin and 250 ng/mL amphotericin B).

Protocol Diagram



Directions for Use

Please read the entire protocol before proceeding. The following protocol is for expanding cultured (P1+) primary human airway epithelial cells (HNECs, HBECs, or HSAECs) in a single T-25 cm^2 flask. If using other cultureware, adjust cell numbers and volumes accordingly.

NOTE: Only use tissue culture-treated cultureware. For expanding HNECs, use tissue culture-treated cultureware coated with collagen I (e.g. Catalog #07001).

1. Plate 1 x 10^5 cells (4 x 10^3 cells/cm^2) in a T-25 cm^2 flask containing 5 mL of room temperature (15 - 25°C) complete PneumaCult™-NGEx Medium (see Preparation section).

   NOTE: The optimal cell plating density may differ depending on the donor.

   NOTE: If starting with cryopreserved cells, thaw cells directly into complete PneumaCult™-NGEx Medium and perform a medium change 24 hours after initial plating.
2. Incubate the cells at 37°C and 5% CO_2. Perform full-medium changes every two days until cells are approximately 60 - 90% confluent and ready to be passaged. This typically takes 2 - 8 days.

   NOTE: The expansion phase may take longer for some donor cell populations. To avoid weekend medium changes, perform a full-medium change with increased volume (i.e. 7.5 mL for a T-25 cm^2 flask) on Friday afternoon, followed by a regular full-medium change on Monday morning.
3. Passage the cells using Animal Component-Free Cell Dissociation Kit, as follows:
   i. Warm sufficient volumes of D-PBS (Without Ca++ and Mg++), complete PneumaCult™-NGEx Medium, ACF Enzymatic Dissociation Solution, and ACF Enzyme Inhibition Solution to room temperature.
   ii. Wash the cells once with 5 mL D-PBS. Discard the wash.
   iii. Add 2 mL of ACF Enzymatic Dissociation Solution and incubate the flask at 37°C for 7 - 12 minutes, or until the cells can be dislodged with gentle tapping of the flask.

   NOTE: The optimal incubation time may differ depending on the donor. After 10 minutes of dissociation, continuously monitor the culture under a microscope.

   NOTE: Trypsin may also be used for dissociation. However, the duration of Trypsin treatment will require optimization.
   iv. Add 2 mL of ACF Enzyme Inhibition Solution to the flask and transfer the cell suspension to a 15 mL conical tube.
   v. Centrifuge the tube at 350 x g for 5 minutes.
   vi. Discard the supernatant and resuspend the cell pellet in 1 - 2 mL of complete PneumaCult™-NGEx Medium.
   vii. Perform a viable cell count using Trypan Blue and a hemocytometer (e.g. Catalog #100-1181).

4. To continue expansion, plate human airway epithelial cells in a new T-25 cm^2 flask and proceed as directed in steps 1 - 3. Alternatively, cells may be plated to initiate downstream differentiation, including air-liquid interface (ALI) cultures and airway organoids, using the following:
   - PneumaCult™-ALI Medium (Catalog #05001)
   - PneumaCult™-ALI-S Medium (Catalog #05050)
   - PneumaCult™ Airway Organoid Kit (Catalog #05060)
   - PneumaCult™ Apical-Out Airway Organoid Medium (Catalog #100-0620)

For complete instructions, refer to the applicable Product Information Sheets, available at www.stemcell.com, or contact us to request a copy.