PneumaCult™-Ex Plus Medium

Serum- and BPE-free medium for expansion of primary human airway epithelial cells

Catalog #05040 500 mL

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Product Description

PneumaCult™-Ex Plus Medium is a serum- and BPE-free cell culture medium that supports more expansion of primary human airway epithelial cells at each passage, compared to other commercially available expansion media. This medium also supports at least two additional passages of cell expansion with better differentiation potential, 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 #05040) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
PneumaCult™-Ex Plus Basal Medium	05041	490 mL	Store at 2 - 8°C.	Stable for 2 years from date of manufacture (MFG) on label.
PneumaCult™-Ex Plus 50X Supplement*	05042	10 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 #
D-PBS (Without Ca++ and Mg++)	37350
Hydrocortisone Stock Solution	07925
Animal Component-Free Cell Dissociation Kit • ACF Enzymatic Dissociation Solution • ACF Enzyme Inhibition Solution	05426
Trypan Blue	07050

Preparation of Complete PneumaCult™-Ex Plus Medium

Use sterile technique to prepare complete PneumaCult™-Ex Plus 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™-Ex Plus 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.

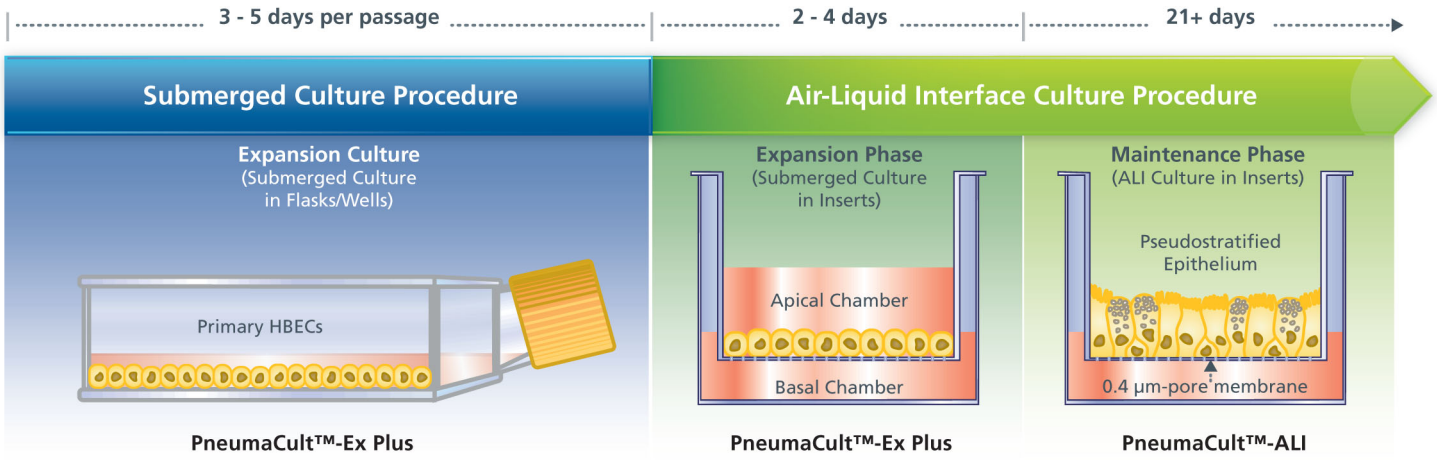
   Once thawed, use the supplement immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquoted supplement, use immediately. Do not re-freeze.

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

   NOTE: If not used immediately, store complete PneumaCult™-Ex Plus Medium at 2 - 8°C for up to 4 weeks. Do not exceed the shelf life of the individual components.

   Complete medium does not contain antibiotics. If desired, they may be added.

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



Directions for Use

Please read the entire protocol before proceeding.

The following protocol is for expanding cultured primary human airway epithelial cells (P1+) 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. If using cells freshly isolated from tissue, additional optimization related to coating and plating density may be required.

1. Plate 1.25 x 10^5 cells (0.5 x 10^4 cells/cm^2) in 5 mL complete PneumaCult™-Ex Plus Medium.

   NOTE: If starting with cryopreserved cells, thaw cells directly into complete PneumaCult™-Ex Plus Medium and perform a medium change 24 hours after initial plating.
2. Incubate cells at 37°C and perform medium changes every two days until cells are approximately 50 - 60% confluent and ready to be passaged. This typically takes 3 - 5 days.

   NOTE: The expansion phase may take longer for some donor cell populations. On weekends, change the medium on Friday afternoon and first thing on Monday morning.
3. Passage cells using the following protocol:
   i. Warm sufficient volumes of D-PBS (Without Ca++ and Mg++), complete PneumaCult™-Ex Plus Medium, and Animal Component-Free Cell Dissociation Kit to room temperature (15 - 25°C).
   ii. Wash cells with 5 mL D-PBS (Without Ca++ and Mg++).
   iii. 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.
   iv. Add 2 mL ACF Enzyme Inhibition Solution and collect cells in a 15 mL 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 complete PneumaCult™-Ex Plus Medium.
   vii. Perform a viable cell count using Trypan Blue and a hemocytometer.

NOTE: For complete instructions on culturing cells at the air-liquid interface in PneumaCult™-ALI Medium or PneumaCult™-ALI-S Medium, refer to the Product Information Sheets available at www.stemcell.com or contact us to request a copy.