PneumaCult™-Ex Medium

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

Catalog #05008 500 mL

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Product Description

PneumaCult™-Ex is a serum- and bovine pituitary extract (BPE)-free cell culture medium that supports rapid expansion of primary human airway epithelial cells. Airway epithelial cells cultured in PneumaCult™-Ex Medium expand rapidly over at least 3 passages while maintaining a cobblestone morphology and uniform expression of the basal cell markers p63 and p75NTR. Cells cultured in PneumaCult™-Ex Medium can be differentiated to form a pseudostratified mucociliary epithelium when cultured at the air-liquid interface (ALI) using PneumaCult™-ALI Medium (Catalog #05001).

Product Information

The following components are sold as a complete kit (Catalog #05008) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
PneumaCult™-Ex Basal Medium	05009	490 mL	Store at 2 - 8°C.	Stable for 12 months from date of manufacture (MFG) on label.
PneumaCult™-Ex 50X Supplement*	05019	10 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
HBSS, Modified (Without Ca++ and Mg++)	37250
Hydrocortisone Stock Solution	07925
Trypsin-EDTA (0.05%)	07910
Soybean Trypsin Inhibitor, ACF	07457
Trypan Blue	07050

Preparation of Reagents and Materials

Use sterile techniques when preparing the following reagents.

Complete PneumaCult™-Ex Medium

The following example is for preparing 500 mL of complete PneumaCult™-Ex Medium (Basal Medium + 50X Supplement + Hydrocortisone Stock Solution). If preparing other volumes, adjust accordingly.

1. Thaw PneumaCult™-Ex 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 of PneumaCult™-Ex 50X Supplement and 0.5 mL of Hydrocortisone Stock Solution to 490 mL of PneumaCult™-Ex Basal Medium. Mix thoroughly.

   NOTE: If not used immediately, store complete PneumaCult™-Ex 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.

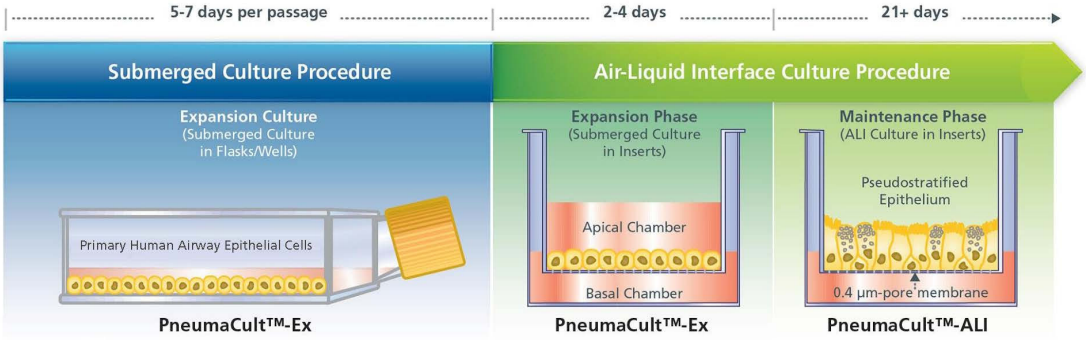
0.025% Trypsin-EDTA

Dilute 0.05% Trypsin-EDTA 1 in 2 in either phosphate-buffered saline (PBS) or HBSS, Modified (Without Ca++ and Mg++).

1 mg/mL Soybean Trypsin Inhibitor

Prepare a 1 mg/mL solution of Soybean Trypsin Inhibitor in HBSS, Modified (Without Ca++ and Mg++).

Schematic of Human Airway Epithelial Cells Cultured in PneumaCult™-Ex 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 2.5 x 10^5 cells (1 x 10^4 cells/cm^2) in 5 mL of complete PneumaCult™-Ex Medium.

   NOTE: If starting with cryopreserved cells, thaw cells directly into complete PneumaCult™-Ex Medium and perform a full medium change 24 hours after initial plating.
2. Incubate cells at 37°C and perform full medium changes every 2 days until cells are approximately 80% confluent and ready to be passaged. This typically takes 5 - 7 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:

   NOTE: Passaging cultures that are < 80% confluent is not recommended.

   i. Warm sufficient volumes of D-PBS (Without Ca++ and Mg++), complete PneumaCult™-Ex Medium, 0.025% Trypsin-EDTA, and 1 mg/mL Soybean Trypsin Inhibitor to room temperature (15 - 25°C).

   ii. Wash cells with 5 mL of D-PBS (Without Ca++ and Mg++).

   iii. Add 2 mL of 0.025% Trypsin-EDTA and incubate at 37°C for 3 - 5 minutes, until cells can be dislodged with gentle tapping of the flask.

   iv. Add 2 mL of 1 mg/mL Soybean Trypsin Inhibitor and collect cells in a 15 mL conical tube (e.g. Catalog #38009).

   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™-Ex 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, refer to the Product Information Sheet (Document #29252) available at www.stemcell.com or contact us to request a copy.