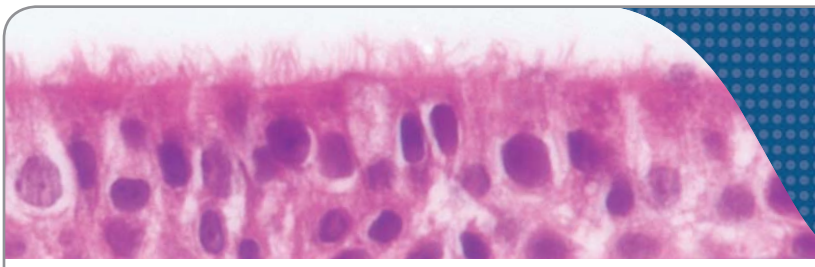

PneumaCult™
for Airway Epithelial Cells

Do Your *Cilia Samba?* Ours Do.
Use PneumaCult™ Media for Airway Epithelial Cells

In vitro models of the human airway using primary nasal, tracheal or bronchial epithelial cells are instrumental in studying basic and applied aspects of airway biology and disease. While simple 2D monolayer (submerged) cultures are commonly used, this method only supports cells with a basal cell phenotype. To obtain in vitro cultures that resemble the in vivo physiology of ciliated, goblet and basal cells arranged in a pseudostratified structure, air-liquid interface (ALI) or other alternative culture methods are required.

The **PneumaCult™ culture system** of defined, bovine pituitary extract (BPE)-free media consists of PneumaCult™-Ex for submerged culture and PneumaCult™-ALI for air-liquid interface culture of primary human bronchial epithelial cells (HBECs; Figure 1). Together, these culture media create an integrated culture system for HBECs that recapitulates the in vivo structure of the airway. Both PneumaCult™-Ex and PneumaCult™-ALI are supplied with detailed protocols, enabling easy preparation of a fully differentiated mucociliary epithelium from undifferentiated HBECs.

Advantages of PneumaCult™ Media

PHYSIOLOGICALLY RELEVANT. Primary HBECs differentiated with PneumaCult™-ALI closely model the human airway

REPRODUCIBLE. PneumaCult™ defined (BPE-free) media formulations maximize experimental reproducibility

USER-FRIENDLY. Uncomplicated components and optimized protocols make PneumaCult™ media easy to use

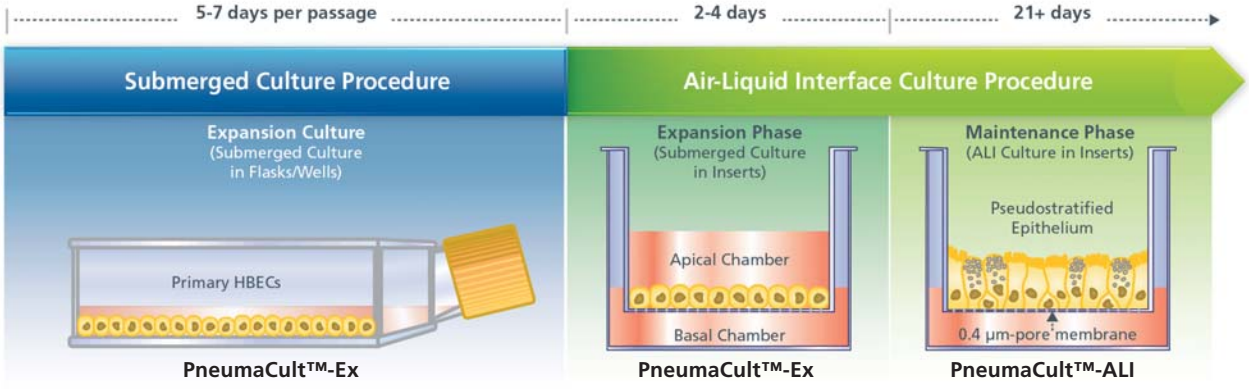

Figure 1. Overview of PneumaCult™ Culture System

Expansion of HBECs in submerged culture is performed with PneumaCult™-Ex. During the 'Expansion Phase' of the ALI culture procedure, PneumaCult™-Ex is applied in both the apical and basal chambers. Upon reaching confluence, the cultures are air-lifted by removing the culture medium and adding PneumaCult™-ALI to the basal chamber only. Differentiated cultures exhibiting a pseudostratified epithelium are obtained following 21-28 days incubation at ALI and can be maintained for extended periods of time (>6 months).

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HBECs cultured in PneumaCult™-Ex have a similar morphology to cells cultured in a commonly used BPE-containing bronchial epithelial growth medium; they exhibit cobblestone morphology (Figure 2) and uniform expression of the basal cell markers p63 and p75^{NTR} (Figure 3). HBECs cultured in PneumaCult™-Ex also display similar expansion rates to those observed in the same commonly used growth medium (Figure 4). Furthermore, HBECs previously cultured in PneumaCult™-Ex can be successfully differentiated with PneumaCult™-ALI to generate a pseudostratified mucociliary epithelium (Figure 5). Primary HBECs differentiated using the PneumaCult™ culture system exhibit the appropriate 3D architecture, with basal cells lining the transwell inserts and ciliated and goblet cells extending to the apical surface. The differentiated cultures start to exhibit widespread ciliary movement 21 to 28 days after air-lift. With appropriate care, these cultures can be maintained for extended periods of time, permitting long-term studies.

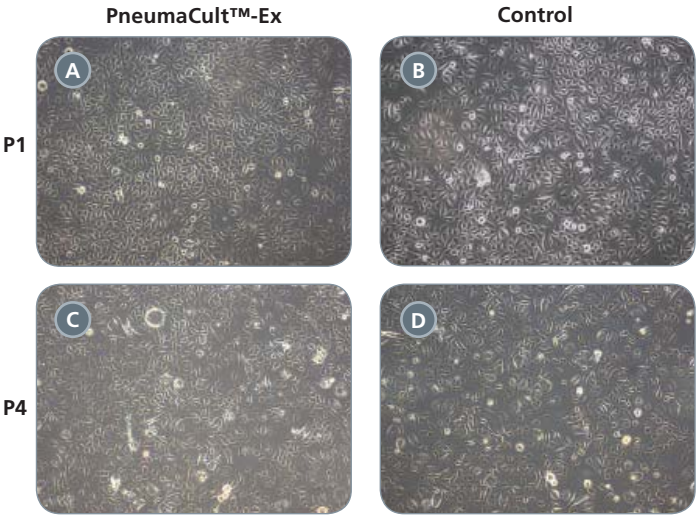

Figure 2. HBECs Cultured in PneumaCult™-Ex Exhibit Cobblestone Morphology

Commercially available, cryopreserved, passage 1 (P1) HBECs were seeded into PneumaCult™-Ex or a Control medium (BEGM™; Lonza). Cells exhibit cobblestone morphology in both culture media, as seen in representative images of confluent cultures 5 days post-seeding (A,B). HBECs cultured for an additional 3 passages in both PneumaCult™-Ex and Control medium continue to expand and retain their normal cobblestone morphology, as shown by representative images of confluent P4 cultures at 7 days post-seeding (C,D). All images were taken through 10X objective.

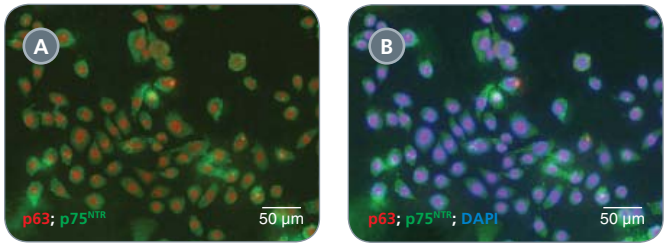

Figure 3. HBECs Cultured in PneumaCult™-Ex Exhibit Uniform Expression of Basal Cell Markers

Passage 3 HBECs cultured in PneumaCult™-Ex demonstrate extensive co-labeling of the basal cell markers p63 (red) and p75^{NTR} (green; A). A representative merged image indicates widespread co-labeling of p63, p75^{NTR} and the nuclear stain DAPI (blue; B).

Differentiated HBEC cultures can also be generated using a sphere culture method.

View sphere cultures at **www.stemcell.com/bronchosphere**

Applications of PneumaCult™

- Studying development and maintenance of the respiratory epithelium
- Modeling respiratory disease
- Studying viral or bacterial respiratory infection
- Preclinical drug development
- Toxicity studies

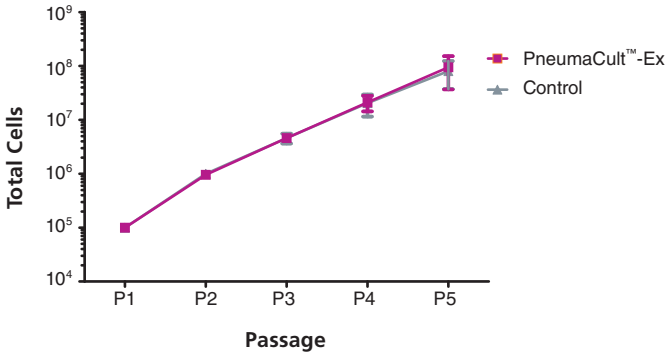

Figure 4. HBECs Cultured in PneumaCult™-Ex Exhibit Comparable Expansion Rates to Cells Cultured in Control Medium

Commercially available, cryopreserved, P1 HBECs were seeded into PneumaCult™-Ex or a Control medium (BEGM™; Lonza). In seven independent donor samples, the average fold expansion over four passages was not significantly different between cells cultured in PneumaCult™-Ex and cells cultured in the Control medium (7.1 ± 1.4 vs. 7.2 ± 1.9; mean ± SD, n = 7, p = 0.9 in paired t-test).

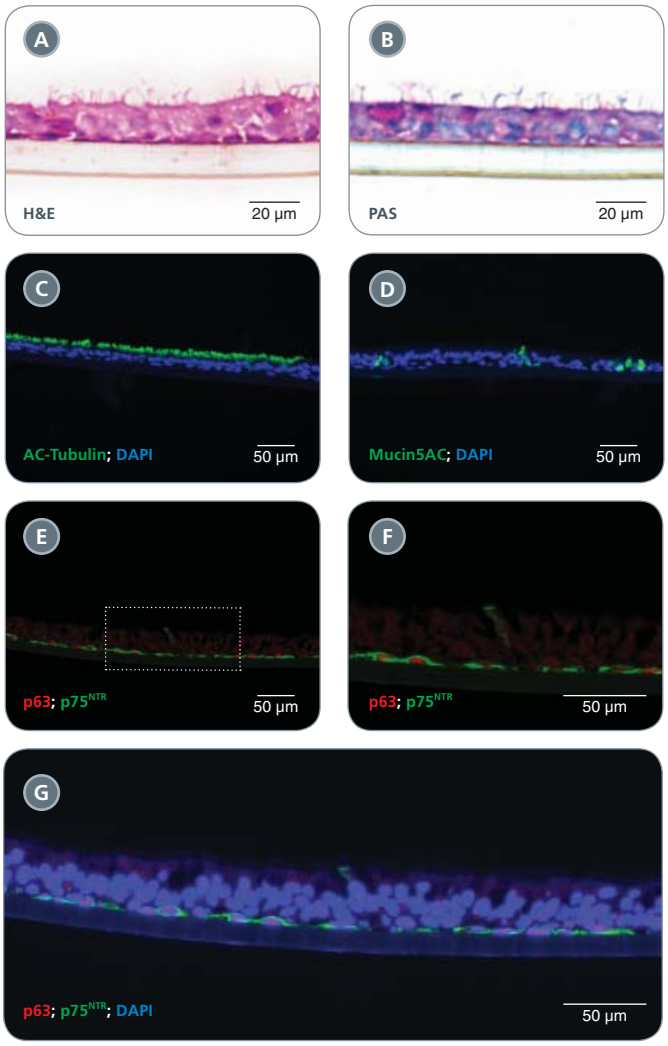

Figure 5. HBECs Cultured in PneumaCult™-Ex Successfully Differentiate into a Pseudostratified Mucociliary Epithelium with PneumaCult™-ALI

Early-passage (P1-3) HBECs cultured in PneumaCult™-Ex successfully differentiate when cultured at air-liquid interface with PneumaCult™-ALI for 28 days. H&E staining revealed the pseudostratified structure of the epithelium with cilia present at the apical surface (A). Periodic acid-Schiff staining demonstrated the presence of goblet cells (B). The presence of ciliated and goblet cells was also demonstrated by immunofluorescence staining of cilia marker acetylated (AC)-Tubulin (green; C) and the goblet cell marker Mucin5AC (green; D). Appropriate positioning of basal cells along the transwell insert was visualized by immunofluorescence staining using the basal cell markers $p75^{NTR}$ (green) and p63 (red; E,F). A representative merged image indicates the apical cells, detected by DAPI alone, positioned along the epithelium and in close contact with the basal cells (detected by DAPI, p63 and $p75^{NTR}$ co-labeling) located along the insert (G).

PneumaCult™
for Bronchial Epithelial Cells

Multimedia Resources

TECHNICAL BULLETIN
Air-Liquid Interface Culture
for Respiratory Research
www.stemcell.com/ALIcultureTB

VIDEO
A Technical Guide to the Successful Mucociliary Differentiation of HBECs with PneumaCult™-ALI
www.stemcell.com/PneumaCultTechVid

WEBSITE
PneumaCult™-ALI Product Information Page
www.stemcell.com/PneumaCult_ALI

Product Information

PRODUCT	QUANTITY	CATALOG #
PneumaCult™-Ex	500 mL	05008
PneumaCult™-ALI	500 mL	05001
0.2% Heparin Sodium Salt in PBS	2 mL	07980
Hydrocortisone	100 mg	07904