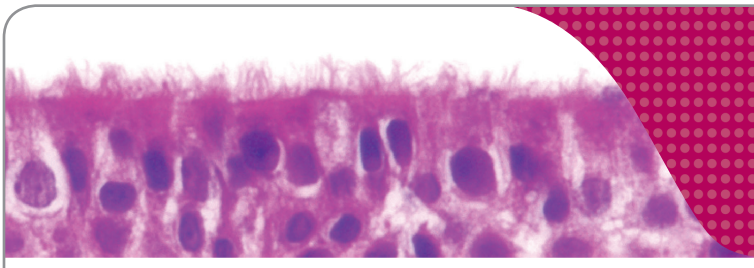

PneumaCult™-Ex Plus: Generate More Airway Epithelial Cells for Extended Passages

Limitation of Current Expansion Media

Current feeder-free expansion media for culturing primary human airway epithelial cells can only support a limited number of passages while maintaining robust mucociliary differentiation potential. Unfortunately, this limitation restricts the number of experiments researchers can perform using primary cells.

PneumaCult™-Ex Plus is a feeder- and BPE-free culture medium that puts an end to this limitation: researchers can expand cells for a higher number of passages during expansion culture, while maintaining mucociliary differentiation potential during the subsequent air-liquid interface (ALI) culture (Figure 1). Ultimately, PneumaCult™-Ex Plus enables researchers to perform more experiments with a single sample.

Why Use PneumaCult™-Ex Plus?

Compared to other commercially available expansion media:

MORE EXPANSION. More population doublings at each passage.

SUSTAINED ALI DIFFERENTIATION POTENTIAL. Maintain morphological and electrophysiological characteristics even after extended passaging.

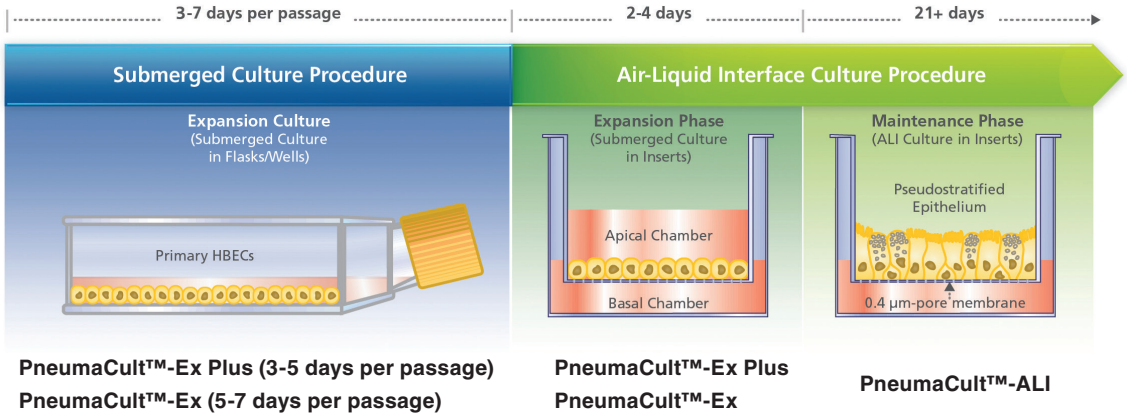

Figure 1. Overview of the PneumaCult™ culture system

Expansion of human bronchial epithelial cells (HBECs) in submerged culture is performed with PneumaCult™-Ex Plus or PneumaCult™-Ex. During the early "Expansion Phase" of the ALI culture procedure, PneumaCult™-Ex Plus or PneumaCult™-Ex is applied to the apical and basal chambers. Upon reaching confluence, the culture is air-lifted by removing the culture medium from both chambers, and adding PneumaCult™-ALI to the basal chamber only. Differentiation into a pseudostratified mucociliary epithelium is obtained following 21-28 days of incubation and can be maintained for more than one year.

How Does PneumaCult™-Ex Plus Compare to Other Commercially-Available Expansion Media?

Study Design

Commercially available primary human bronchial epithelial cells (HBECs) at passage 1 (P1) were thawed and seeded into T-25cm^2 flasks containing PneumaCult™-Ex Plus, PneumaCult-Ex™, or Bronchial Epithelial Growth Media. At each passage, cells were enzymatically dissociated and passaged once cultures reached approximately 50-70% confluence, followed by differentiation in ALI culture using PneumaCult™-ALI.

Expansion: More Population Doublings at Each Passage

HBECs cultured in PneumaCult™-Ex Plus experience at least two more population doublings compared to those cultured in PneumaCult™-Ex or Bronchial Epithelial Growth Media (Figure 2). PneumaCult™-Ex Plus cultures are characterized by smaller and more tightly packed cells (Figure 3) that express higher levels of basal cell markers CD49f and CD271 (Figures 4 and 5). The maintenance of stem-like basal cells in PneumaCult™-Ex Plus permits better ALI differentiation potential even after extended passaging.

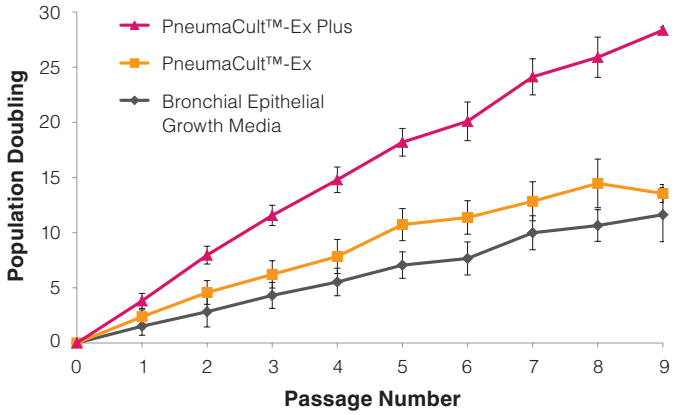

Figure 2. HBECs cultured in PneumaCult™-Ex Plus have a faster expansion rate compared to those cultured in PneumaCult™-Ex and Bronchial Epithelial Growth Media

Commercially available, cryopreserved P1 HBECs were seeded into PneumaCult™-Ex Plus, PneumaCult™-Ex, or Bronchial Epithelial Growth Media. Cells cultured in PneumaCult™-Ex Plus have a significantly higher proliferation rate over 9 passages compared to those maintained in either control medium (n=6).

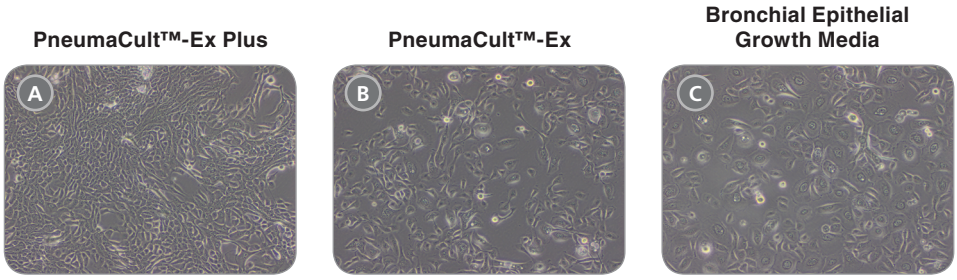

Figure 3. Representative morphology of HBECs

Representative live culture images for P4 HBECs cultured in PneumaCult™-Ex Plus, PneumaCult™-Ex, or Bronchial Epithelial Growth Media. Cells cultured in PneumaCult™-Ex Plus (A) are smaller and more tightly packed than those cultured in PneumaCult™-Ex (B) or Bronchial Epithelial Growth Media (C). All images were taken using a 10X objective.

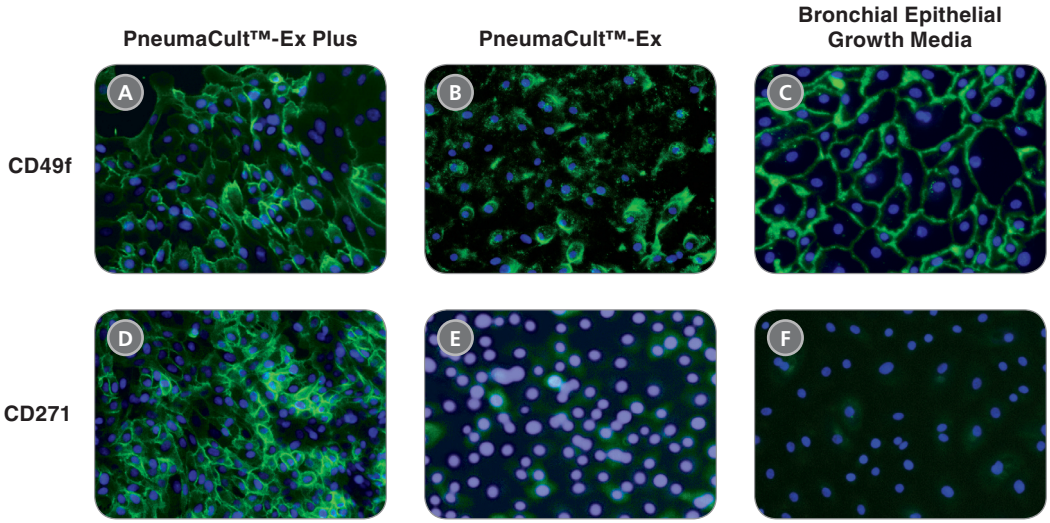

Figure 4. HBECs cultured in PneumaCult™-Ex Plus maintain widespread expression of the basal cell markers CD49f and CD271

Immunocytochemistry detection of basal cell markers - CD49f (A, B, and C) and CD271 (D, E, and F) - for P4 HBECs cultured in PneumaCult™-Ex Plus (A and D), PneumaCult™-Ex (B and E), and Bronchial Epithelial Growth Media (C and F). All images were taken using a 10X objective.

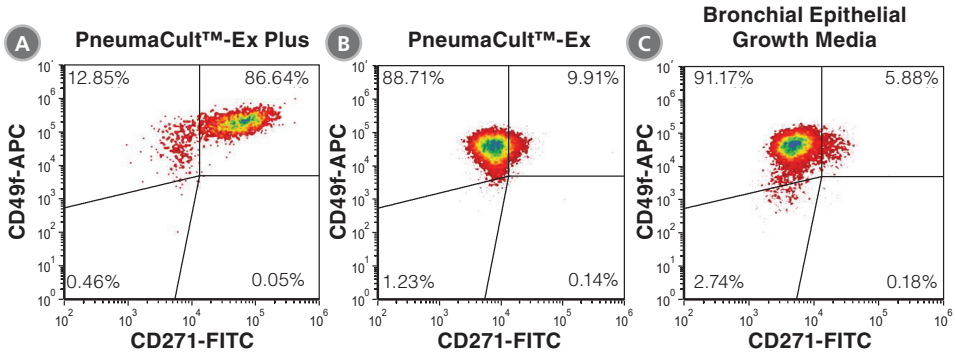

Figure 5. HBECs cultured in PneumaCult™-Ex Plus have a higher proportion of CD271$^+$CD49f$^+$ cells

P4 HBECs cultured in PneumaCult™-Ex Plus (A), PneumaCult™-Ex (B), and Bronchial Epithelial Growth Media (C) were characterized by flow cytometry to detect expression of the basal cell markers CD49f and CD271. HBECs cultured in PneumaCult™-Ex Plus (A) have a higher proportion of cells coexpressing CD49f and CD271, compared to those cultured in PneumaCult™-Ex (B) and Bronchial Epithelial Growth Media (C).

Differentiation: Maintaining ALI Differentiation Potential Even After Extended Passaging

Differentiation potential was assessed by seeding the HBECs expanded in different expansion media to ALI culture using PneumaCult™-ALI.

Morphology

ALI cultures from early passages of HBECs have a similar morphology regardless of the type of expansion medium. However, beginning at P5, HBECs cultured in PneumaCult™-Ex Plus demonstrate a clear advantage over those cultured in either control medium, and exhibit better pseudostratified mucociliary differentiation indicated by higher expression of the cilia marker AC-tubulin (red) and goblet cell marker Muc5AC (green) (Figure 6).

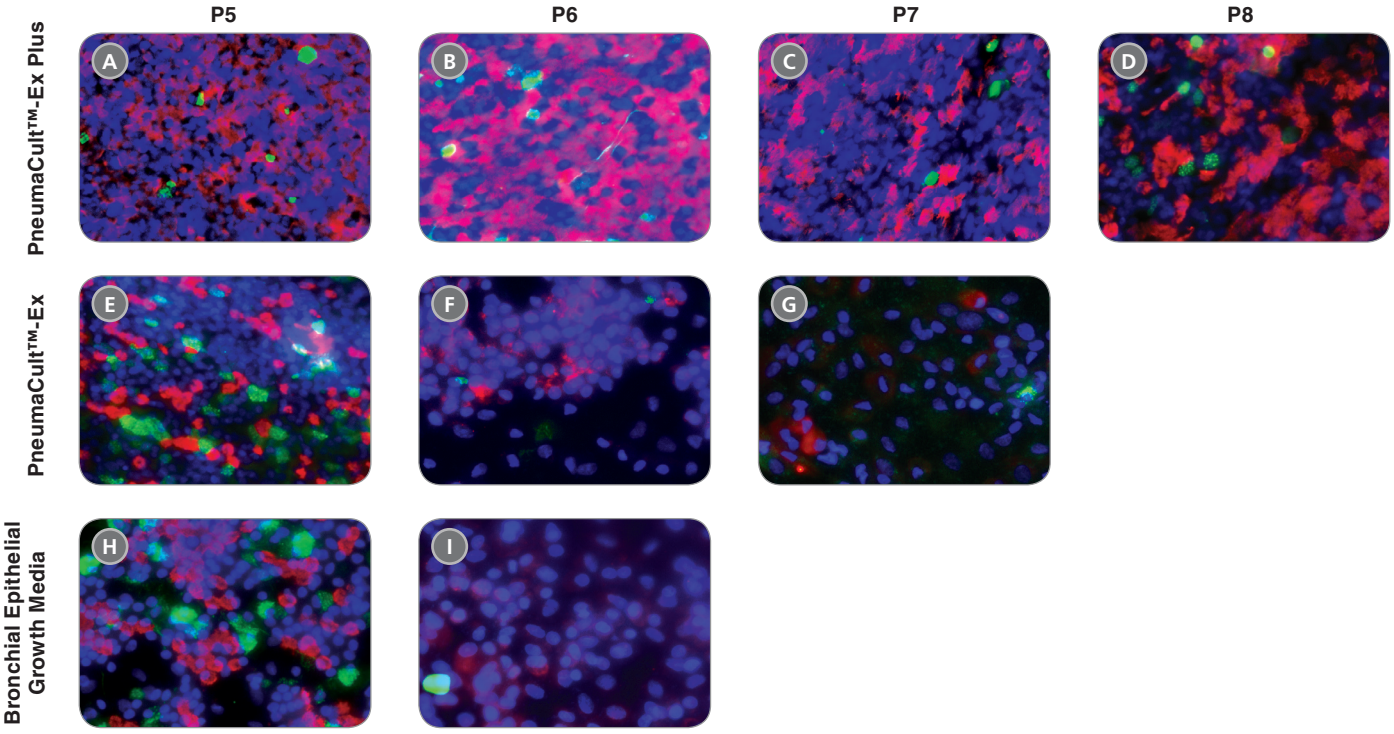

Figure 6. HBECs cultured in PneumaCult™-Ex Plus differentiate into a pseudostratified mucociliary epithelium at later passages with the use of PneumaCult™-ALI

P4 HBECs were seeded and passaged using PneumaCult™-Ex Plus, PneumaCult™-Ex, or Bronchial Epithelial Growth Media, followed by ALI differentiation at each passages (P5-8) with the use of PneumaCult™-ALI. The ALI cultures at 28 days post air-lift were fixed and stained with antibodies for cilia marker AC-tubulin (red) and the goblet cell marker Muc5AC (green). The nuclei are counterstained with DAPI (blue). All images were taken using a 20X objective.

Electrophysiological Function

ALI cultures initiated with HBECs expanded in different expansion media were characterized electrophysiologically to examine Trans-Epithelial Electrical Resistance (TEER) and Short Circuit Current (Isc) using a Ussing Chamber. While TEER measures the integrity and health of the confluent epithelial layer, Isc measures the active transport of ions across the epithelial cell layer. After 28 days of ALI differentiation, HBECs originally expanded in PneumaCult™-Ex Plus have better barrier integrity than those expanded in either control medium, indicated by higher TEER values at each passage (Figure 7A). They also have higher ion transport activities across the epithelial cell layer, indicated by higher drug-responsiveness specifically for the epithelial sodium channel (ENaC) and Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) channel (Figure 7B).

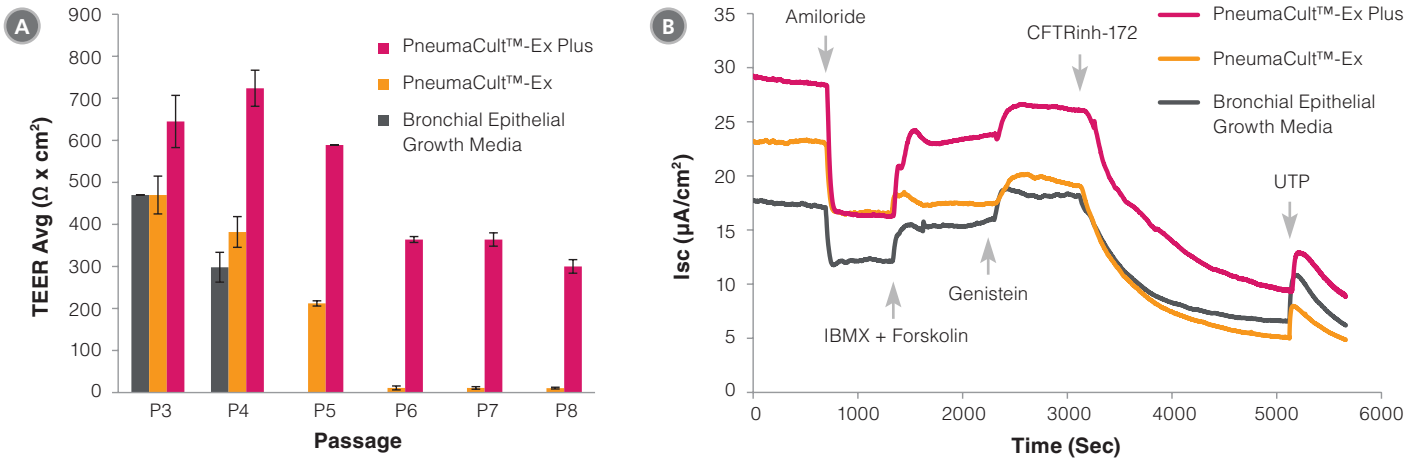

Figure 7. Electrophysiological characterization of differentiated HBECs (P4) that were expanded in PneumaCult™-Ex Plus, PneumaCult™-Ex, and Bronchial Epithelial Growth Media

TEER (A) and representative characterization of the ion channel activities (B) for ALI cultures at 28 days post air-lift using HBECs expanded in PneumaCult™-Ex Plus, PneumaCult™-Ex, or Bronchial Epithelial Growth Media. Amiloride: ENaC inhibitor. IBMX and Forskolin: CFTR activators. Genistein: CFTR potentiator. CFTRinh-172: CFTR inhibitor. UTP: Calciumactivated Chloride channels (CaCCs) activator. All ALI differentiation cultures were performed using PneumaCult™-ALI.

Reference

1. Rock JR et al. (2009) Basal cells as stem cells of the mouse trachea and human airway epithelium. PNAS (106): 12771-12775.