



PULMONARY RESEARCH

Culture Media and Tools for
Human Airway and Alveolar
Epithelial Cells



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In Vitro Human Airway and Alveolar Model Systems

In vitro models of the human airway using primary nasal, tracheal, bronchial, or alveolar cells are instrumental in studying basic and applied aspects of airway biology and disease. Traditional two-dimensional (2D) submerged culture systems only support cells with a basal cell phenotype, limiting their homology to in vivo human airway epithelium. Air-liquid interface (ALI) culture is an established in vitro model that mimics the complex morphological and functional characteristics of the in vivo human airway.^{1,2} For example, tracheal and bronchial epithelial cells cultured at the ALI differentiate and form a pseudostratified epithelium with barrier functions and representative cell heterogeneity.^{1,2} ALI cultures of primary cells from donors with respiratory diseases such as asthma, cystic fibrosis (CF), and chronic obstructive pulmonary disease (COPD) exhibit in vivo disease characteristics.^{3,4}

Three-dimensional (3D) human airway and alveolar organoids represent another versatile system for modeling the human airway. These systems accurately replicate the histological and functional aspects of the in vivo tissue and their cellular morphology is physiologically similar to the human airway and alveolar epithelium. By developing these structures and functions, these organoid models emulate the in vivo physiological or pathological environment, making them useful for modeling respiratory diseases, drug discovery and toxicity studies, and observing the functions of the airway and alveolar epithelium. Compared to ALI cultures, human airway and alveolar organoids are generated using a cell culture insert-free protocol, facilitating high-throughput drug screening.

PneumaCult™ Culture System

The PneumaCult™ culture system consists of optimized cell culture media for modeling the human airway and alveoli in vitro. Various airway and alveolar systems can be achieved as follows:

- Primary human bronchial epithelial cells (HBECs) or human small airway epithelial cells (HSAECs) can be expanded in submerged culture with PneumaCult™-Ex Plus Medium, and ALI cultures of differentiated HBECs or HSAECs can be achieved with PneumaCult™-ALI Medium or PneumaCult™-ALI-S Medium, respectively.
- For differentiation as 3D organoids, PneumaCult™ Airway Organoid Kit or PneumaCult™ Apical-Out Airway Organoid Medium are recommended.
- To model the human alveolar physiology with an in vitro system that recapitulates key features of ATII and ATI cells in vivo, PneumaCult™ Alveolar Organoid Media can be used.

This robust and fully integrated culture system is a valuable tool for basic respiratory research, toxicity studies, and drug development. PneumaCult™ media is also compatible with other species such as ferret,⁵ mouse,⁶ pig,⁷ rat,⁸ and rhesus macaque.⁹

Why Use PneumaCult™ for In Vitro Airway and Alveolar Cultures?

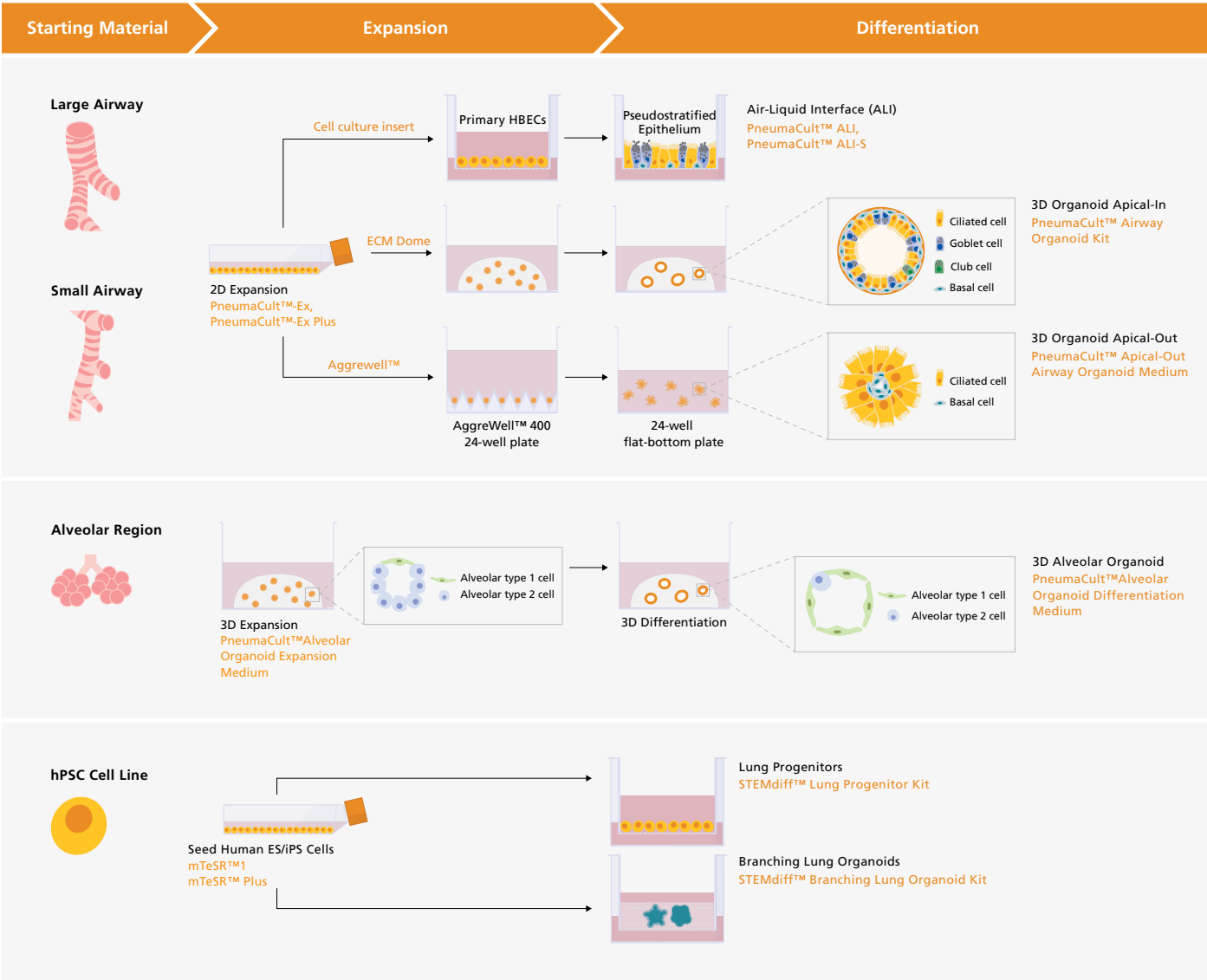
PHYSIOLOGICALLY RELEVANT. Model the human airway with in vitro model systems that closely recapitulate what is observed in vivo.

REPRODUCIBLE. Maximize experimental reproducibility and your confidence with rigorous raw material screening and extensive quality control testing.

COMPLETE. Expand and differentiate human airway epithelial cells with this complete medium system for airway cultures.

USER-FRIENDLY. Get started now with convenient formats and easy-to-use protocols.

STEMCELL Technologies' Products for In Vitro Airway and Alveolar Cultures



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PRODUCT FINDER
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www.stemcell.com/Pulmonary-Product-Finder

Expansion of Human Primary Airway Epithelial Cells

PneumaCult™-Ex Plus Medium

Current feeder-free expansion media for culturing human airway epithelial cells (HAECs) 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 Medium is a feeder- and BPE-free culture medium that puts an end to this limitation. Using PneumaCult™-Ex Plus Medium, researchers can expand cells for a higher number of passages during expansion culture, while maintaining mucociliary differentiation potential during the subsequent ALI or airway organoid culture. Ultimately, PneumaCult™-Ex Plus Medium enables researchers to perform more experiments with a single sample.

Why Use PneumaCult™-Ex Plus Medium?

EFFICIENT. Obtain more population doublings at each passage.

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

RELIABLE. Get consistent performance with a defined, serum- and BPE-free formulation.

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

To compare the performance of PneumaCult™ against other media, commercially available primary human bronchial epithelial cells (HBECs) at passage 1 (P1) or human small airway epithelial cells (HSAECs) at passage 2 (P2) were thawed and seeded into T-25 cm² flasks containing PneumaCult™-Ex Plus Medium, PneumaCult™-Ex Medium, or commercially available bronchial epithelial growth medium or small airway epithelial cell growth medium. At each passage, cells were enzymatically dissociated and passaged once cultures reached approximately 50–70% confluence.

Expansion Rate: HBECs cultured in PneumaCult™-Ex Plus Medium experience at least two more population doublings compared to those cultured in Bronchial Epithelial Growth Medium (Figure 1). Similarly, HSAECs cultured in PneumaCult™-Ex Plus Medium exhibit a higher rate of proliferation (Figure 2) compared to those cultured in Small Airway Epithelial Cell Growth Medium. HBEC cultures growing in PneumaCult™-Ex Plus Medium are characterized by smaller and more tightly packed cells (Figures 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 Medium permits better ALI differentiation potential even after extended passaging.

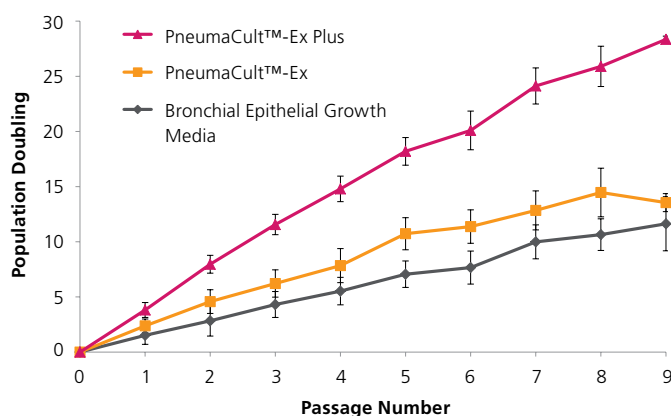


Figure 1. HBECs Cultured in PneumaCult™-Ex Plus Have a Faster Expansion Rate Compared to Those Cultured in PneumaCult™-Ex and Bronchial Epithelial Growth Media

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).

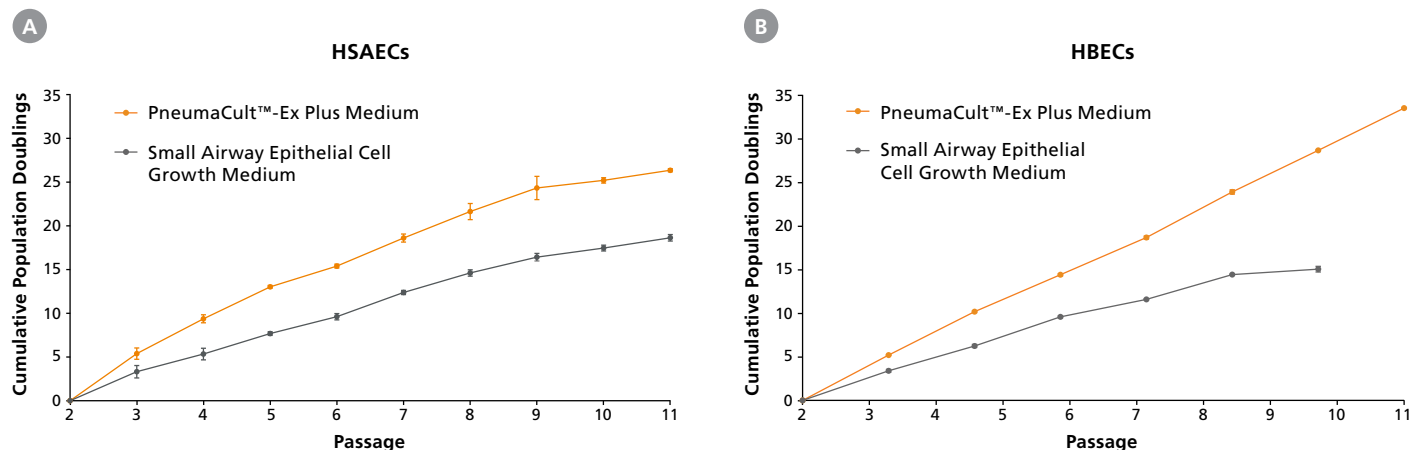


Figure 2. Human Small Airway Epithelial Cells and Human Bronchial Epithelial Cells Grow at a Higher Rate During Expansion When Cultured in PneumaCult™-Ex Plus Medium

(A) HSAECs and (B) HBECs cultured in PneumaCult™-Ex Plus Medium exhibited higher proliferation rate at every passage compared with cells cultured in small airway epithelial cell growth medium. Cryopreserved HSAECs were obtained commercially at Passage 2 while HBECs were obtained at Passage 1.

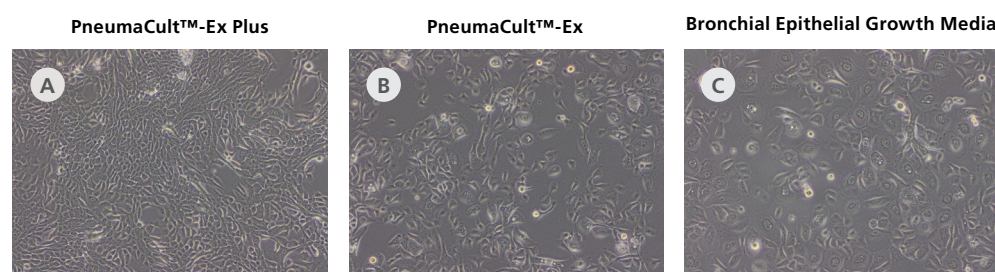


Figure 3. Representative Morphology of HBECs Cultured in PneumaCult™ and Bronchial Epithelial Growth Media

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

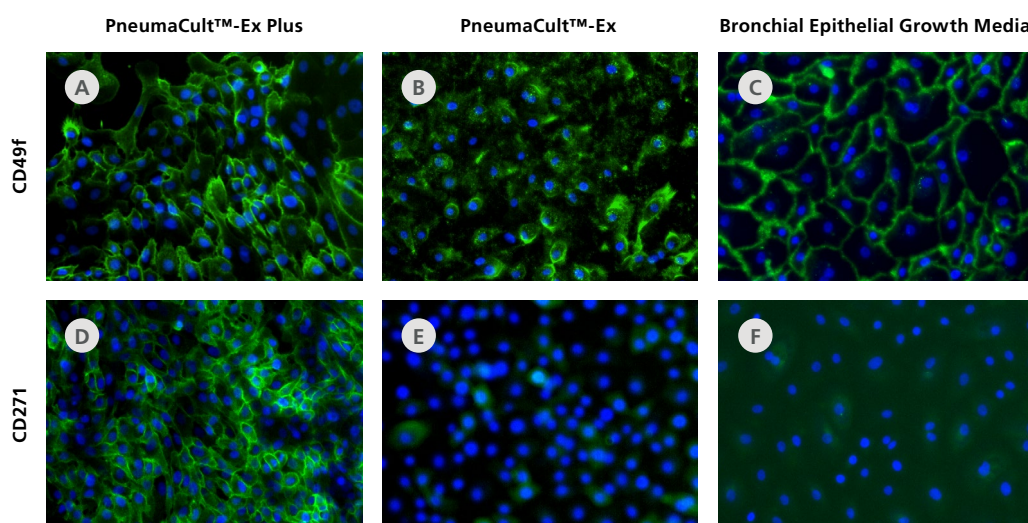


Figure 4. HBECs Cultured in PneumaCult™-Ex Plus Maintain Widespread Expression of the Basal Cell Markers CD49f and CD271

Immunocytochemistry detection of basal cell markers (A-C) CD49f and (D-F) CD271 for P4 HBECs cultured in (A and D) PneumaCult™-Ex Plus, (B and E) PneumaCult™-Ex, and (C and F) bronchial epithelial growth media. Cells cultured in each medium demonstrate comparable expression of both markers. All images were taken using a 10X objective.

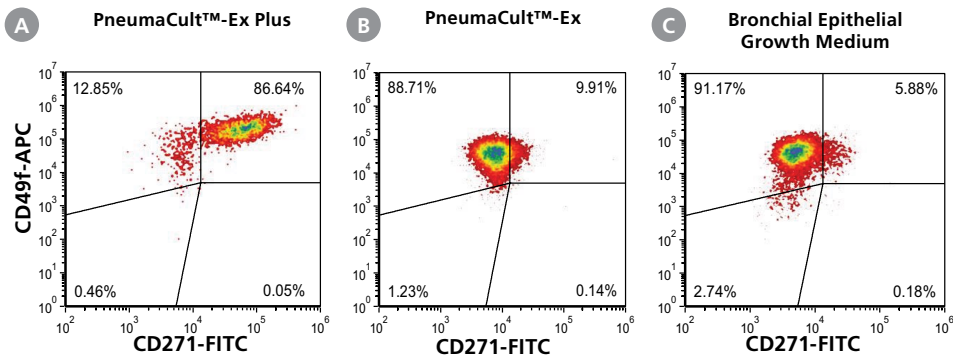


Figure 5. HBECs Cultured in PneumaCult™-Ex Plus Have a Higher Proportion of CD271⁺CD49f⁺ Cells

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

Morphology: ALI cultures from early passages of HBECs have a similar morphology regardless of the type of expansion medium. However, beginning at Passage 5 (P5), HBECs cultured in PneumaCult™-Ex Plus Medium demonstrate a clear advantage over those cultured in bronchial epithelial growth medium, and exhibit better pseudostratified mucociliary differentiation, indicated by higher expression of markers for ciliated cells and goblet cells (Figure 6).

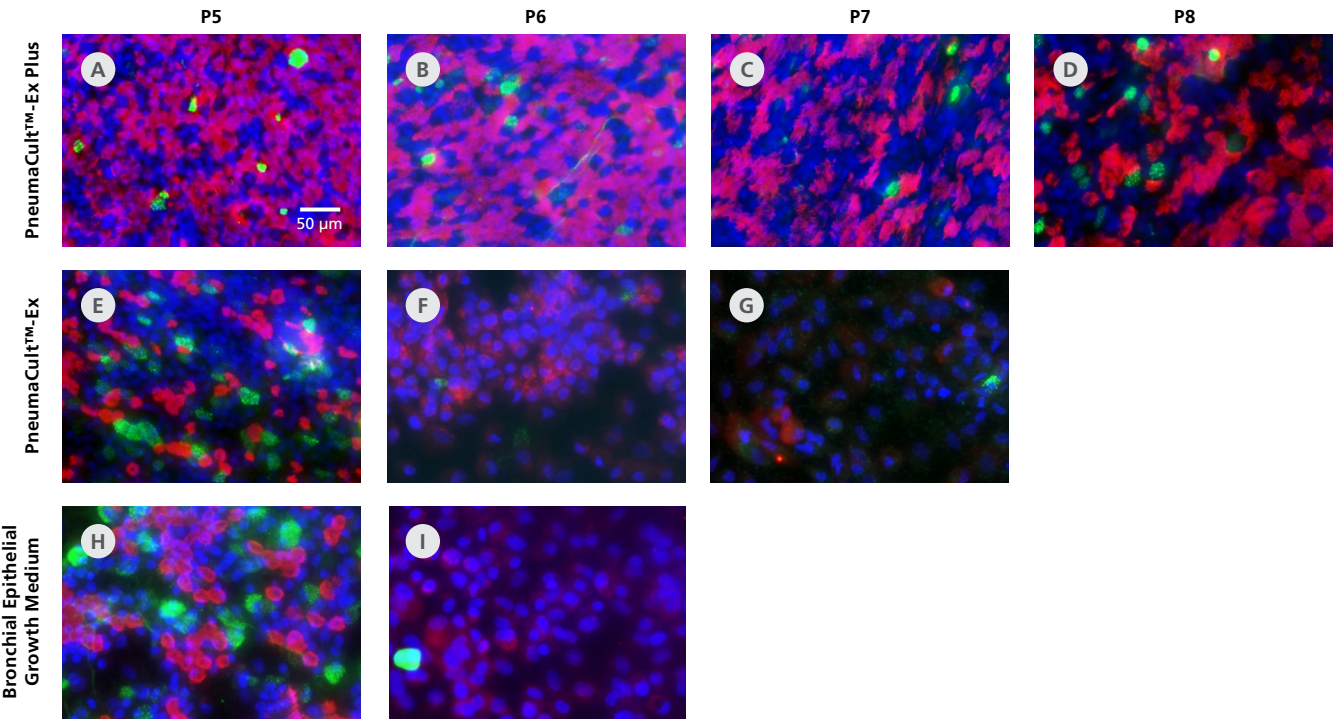


Figure 6. HBECs Cultured in PneumaCult™-Ex Plus Differentiate into a Pseudostratified Mucociliary Epithelium at Later Passages with the Use of PneumaCult™-ALI

P4 HBECs were seeded and passaged using PneumaCult™-Ex Plus, PneumaCult™-Ex, or bronchial epithelial growth media, followed by ALI differentiation at each passage (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), which measures the integrity and health of the confluent epithelial layer, and Short Circuit Current (Isc), which measures the active transport of ions across the epithelial cell layer and is determined using an Ussing Chamber. After 28 days of ALI differentiation, HBECs originally expanded in PneumaCult™-Ex Plus Medium showed better barrier integrity than those expanded in Bronchial Epithelial Growth Medium and PneumaCult™-Ex, indicated by higher TEER values at each passage (Figure 7A). They also exhibited higher ion transport activities across the epithelial cell layer, indicated by higher drug-responsiveness, specifically for the epithelial sodium channel (ENaC) and CFTR channel (Figure 7B).

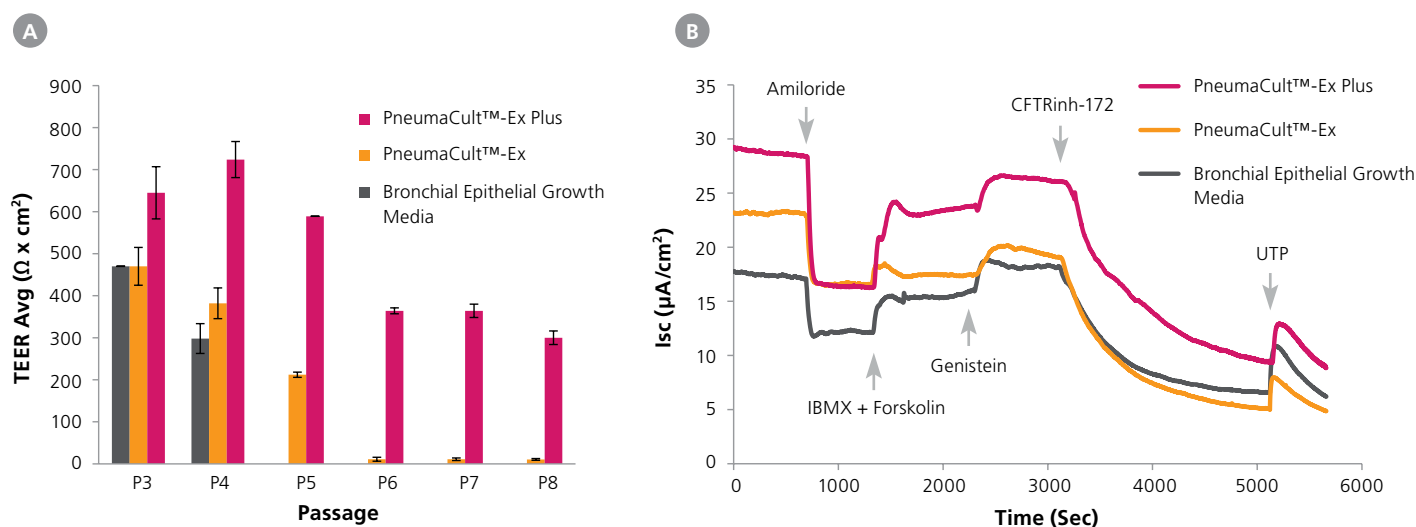


Figure 7. Transepithelial Electrical Resistance and Ion Channel Activity Is Improved in HBECs Cultured in PneumaCult™-Ex Plus

(A) Transepithelial electrical resistance (TEER) and (B) representative characterization of the ion channel activities 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.



PROTOCOL

Expansion of HBECs

www.stemcell.com/HBEC-Expansion



PROTOCOL

Measure TEER in ALI cultures

www.stemcell.com/TEER-Protocol

Differentiation of Human Primary Airway Epithelial Cells at the Air-Liquid Interface

Air-Liquid Interface (ALI) Culture Systems

ALI cultures are one of the most well-characterized models of the *in vivo* human airway. Primary human bronchial epithelial cells (HBECs) or human small airway epithelial cells (HSAECs) can be expanded in submerged culture with PneumaCult™-Ex or PneumaCult™-Ex Plus Medium, and ALI cultures of differentiated HBECs or HSAECs can be achieved with PneumaCult™-ALI Medium or PneumaCult™-ALI-S Medium, respectively.

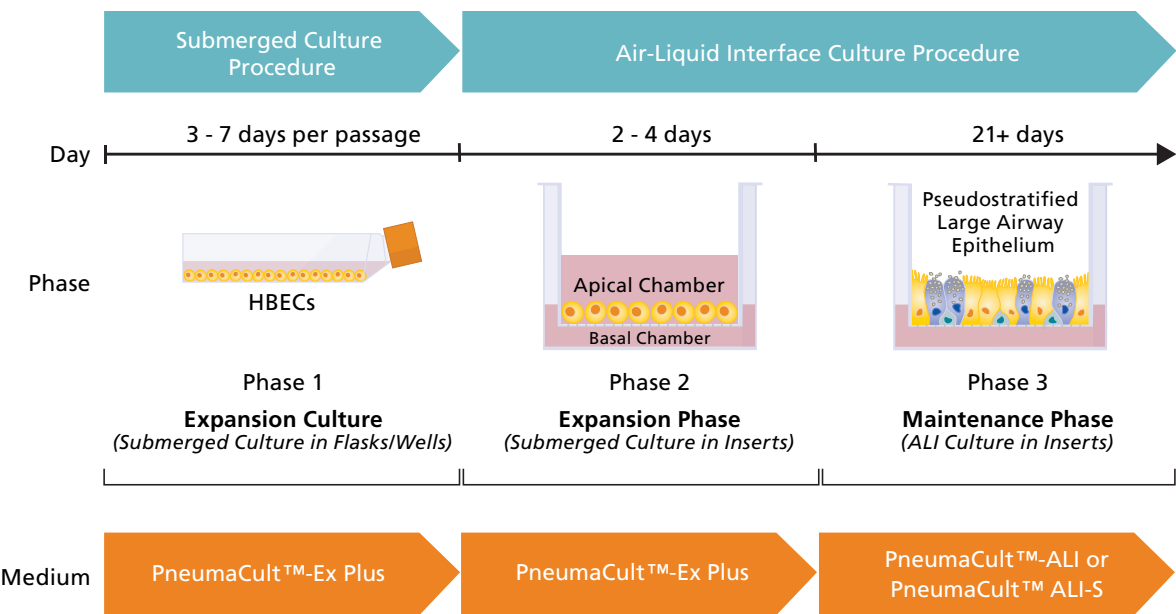


Figure 8. Overview of the PneumaCult™ Air-Liquid Interface Culture System

HBECs or HSAECs are first expanded in submerged culture using PneumaCult™-Ex Plus Medium. In the “Expansion Phase” of the ALI culture procedure, PneumaCult™-Ex Plus Medium is applied to the apical and basal chambers. Upon reaching confluence, the culture is air-lifted by removing the medium from both chambers, and adding PneumaCult™-ALI Medium (for HBECs) or PneumaCult™-ALI-S Medium (for HSAECs) to the basal chamber only. Differentiation into a pseudostratified (large airway) or cuboidal (small airway) mucociliary epithelium is achieved following 21 - 28 days of incubation and can be maintained for more than one year.

PneumaCult™-ALI Medium

PneumaCult™-ALI Medium is a serum- and BPE-free medium for the culture of HBECs at the ALI. HBECs expanded in PneumaCult™-Ex Plus Medium and differentiated in PneumaCult™-ALI Medium undergo extensive mucociliary differentiation to form a pseudostratified epithelium (Figure 9A) that exhibits morphological and functional characteristics similar to those of the human airway in vivo (Figure 9B).

After complete differentiation, an ALI culture of HBECs differentiated in PneumaCult™-ALI Medium expresses key markers characteristic of the large airway (tracheobronchial epithelium), including ciliated cells, mucus-secreting goblet cells, and apical tight junctions (Figure 10).

An important function of the large airway in vivo is to act as a protective barrier against inhaled insults. The same epithelial barrier function has been confirmed in ALI cultures generated using PneumaCult™-ALI Medium by the expression of tight junction proteins and the development of high TEER (Figure 9A).

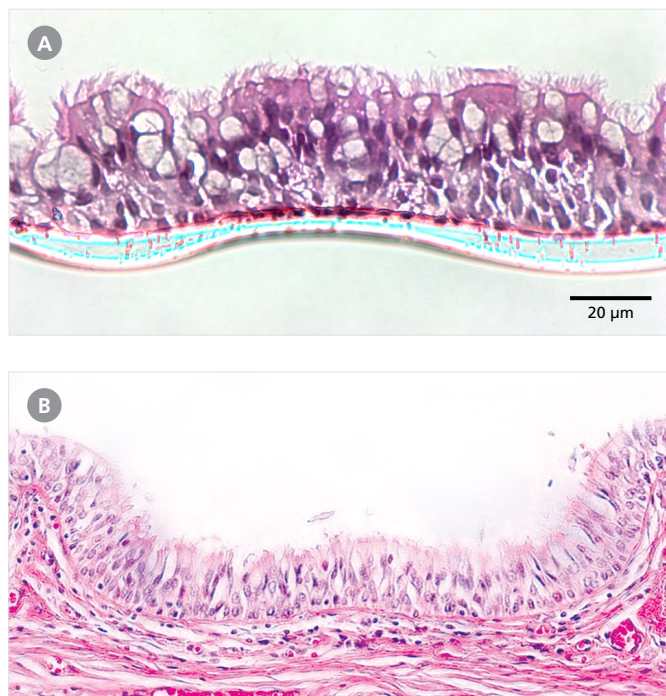


Figure 9. Primary Human Bronchial Epithelial Cells Cultured at the Air-Liquid Interface Recapitulate the In Vivo Bronchial Epithelium

Hematoxylin and eosin (H&E) staining reveals that cells expanded in PneumaCult™-Ex Plus Medium and differentiated at the ALI in (A) PneumaCult™-ALI Medium form a pseudostratified epithelium that is representative of (B) the in vivo bronchial epithelium.

Why Use PneumaCult™-ALI Medium?

PHYSIOLOGICAL. Generate a pseudostratified epithelium that closely resembles the human airway in vivo.

REPRODUCIBLE. Maximize experimental reproducibility with a defined formulation.

USER-FRIENDLY. Get started now with an optimized and easy-to-follow protocol.

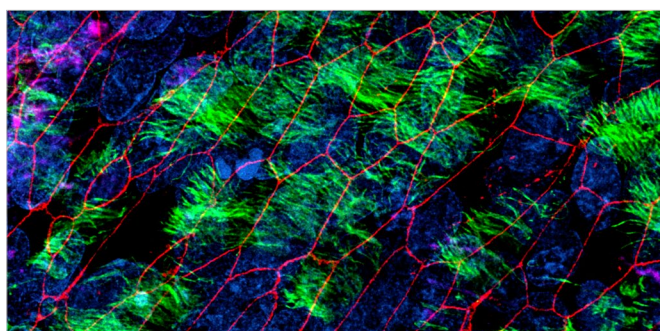


Figure 10. PneumaCult™-ALI Medium Enables Differentiation of Human Bronchial Epithelial Cells to Cell Types Present in the Large Airway Epithelium

Confocal image of whole mount immunostained ALI culture showing Passage 2 (cystic fibrosis) CF HBECs expanded in PneumaCult™-Ex Plus Medium and differentiated at the ALI using PneumaCult™-ALI Medium, after 28 days. The ALI culture was fixed and stained with antibodies for ciliated cells (AC-tubulin; green), Zo-1 (cell junction; red), and goblet cells (Muc5AC; pink). The nuclei were counterstained with DAPI (blue). Image was taken using a 63X objective.



PROTOCOL

Differentiation of HBECs

www.stemcell.com/HBEC-Differentiation

PneumaCult™-ALI-S Medium

To date, clinical and basic science applications of ALI culture have focused primarily on modeling the human bronchial epithelium, which is the site of disruption for numerous respiratory diseases. However, increasing evidence implicates the small airway epithelium (SAE), located after the 8th generation bronchi, in the pathogenesis of major lung disorders such as COPD, asthma, idiopathic pulmonary fibrosis, cystic fibrosis, and most lung cancers.^{17,18} Compared with the pseudostratified epithelium of the large airway, the SAE is characterized by a thin, single-celled cuboidal epithelium of basal, secretory, ciliated, and surfactant protein-positive cells.^{17,18} Furthermore, the cell population in the small airway differs in proportion and biological properties, consisting of more ciliated cells and secretoglobin-producing club cells, but fewer mucus-producing goblet cells.^{17,19} Given the regional differences between the large and small airways, physiologically relevant small airway research requires specific culture conditions to support in vitro modeling of the distinct biology and pathophysiology of the SAE.

PneumaCult™-ALI-S Medium is a serum- and BPE-free differentiation medium optimized for the culture of HSAECs at the ALI. HSAECs expanded in PneumaCult™-Ex Plus Medium and cultured in PneumaCult™-ALI-S Medium undergo extensive mucociliary differentiation to form a thin, cuboidal epithelium (Figure 11) that exhibits morphological and functional characteristics representative of the in vivo human small airway. Together, PneumaCult™-ALI-S Medium and PneumaCult™-Ex Plus Medium constitute a fully integrated serum- and BPE-free culture system for in vitro human small airway modeling.

After complete differentiation, an ALI culture of HSAECs differentiated in PneumaCult™-ALI-S Medium expresses key markers characteristic of the small airway epithelium, including ciliated cells, club cells, and secretory proteins (Figure 12 and 13).



PROTOCOL

Perform ICC staining of ALI cultures
www.stemcell.com/ALI-ICC-Staining



VIDEO

Correlate TEER values with ALI culture morphology
www.stemcell.com/TEER-Video

Why Use PneumaCult™-ALI-S Medium?

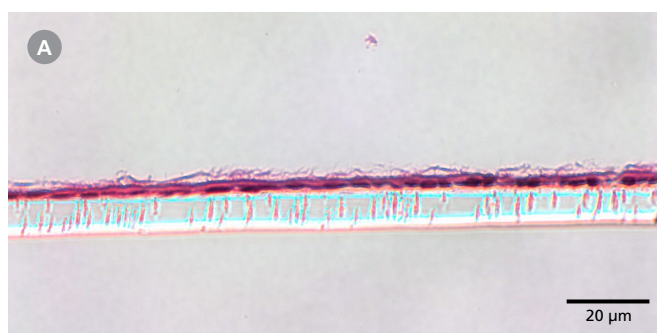
REGIONAL SPECIFICITY. Differentiate to ALI cultures with morphology and cell-type ratio representative of the small airway epithelium.

RELEVANT. Generate in vitro small airway epithelial cell cultures that model the in vivo human small airway.

COMPLETE. Expand, maintain, and differentiate small airway epithelial cells by using with PneumaCult™-Ex Plus Medium.

REPRODUCIBLE. Maximize experimental reproducibility with an optimized formulation.

PneumaCult™-ALI-S Medium



PneumaCult™-ALI Medium

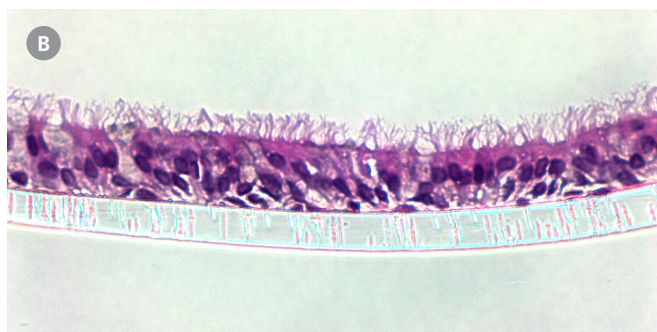


Figure 11. Human Small Airway Epithelial Cells Cultured at the Air-Liquid Interface Using PneumaCult™-ALI-S Medium Recapitulate the Small Airway Epithelium

H&E staining of HSAECs expanded in PneumaCult™-Ex Plus Medium and differentiated in (A) PneumaCult™-ALI-S Medium or (B) PneumaCult™-ALI Medium at Passage 3, after 28 days. HSAECs differentiated at the ALI in PneumaCult™-ALI-S Medium formed a thin, cuboidal epithelial layer representative of the in vivo small airway epithelium. The ALI cultures were fixed, paraffin-embedded, sectioned, and stained with H&E. All images were taken using a 40X objective. Insert membrane was 10 μm in thickness. Scale bar = 20 μm.

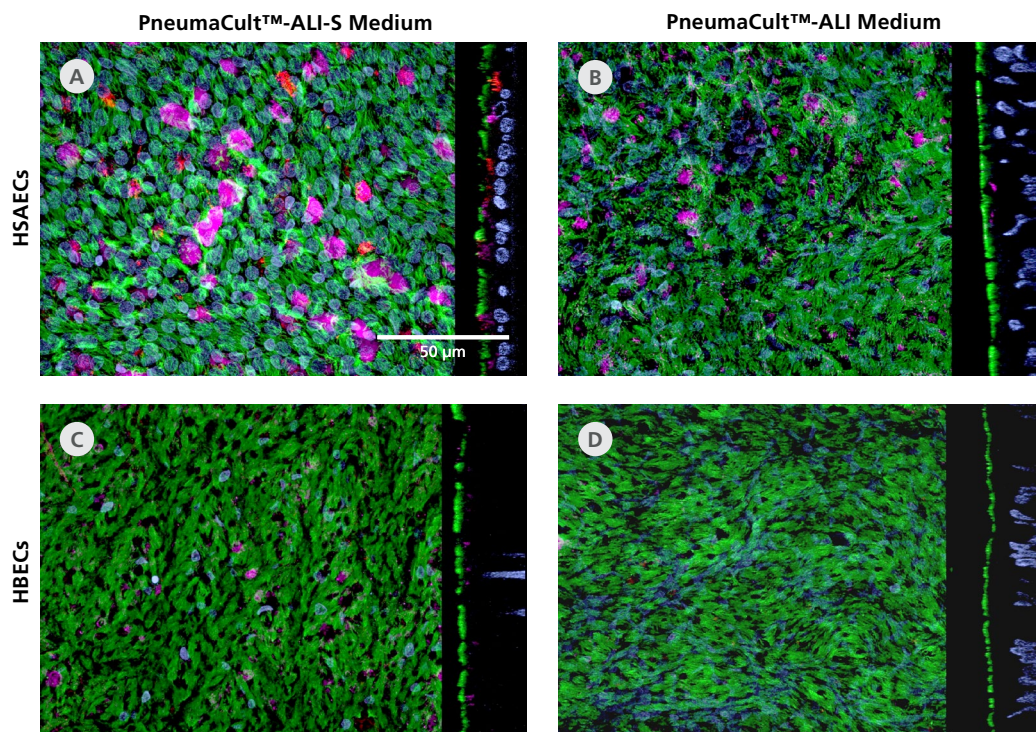


Figure 12. PneumaCult™-ALI-S Medium Enables Differentiation of Human Small Airway Epithelial Cells to Cell Types Present in the Small Airway Epithelium

Confocal images of whole mount immunostained ALI cultures showing (A,B) HSAECs and (C,D) HBECs expanded in PneumaCult™-Ex Plus Medium and differentiated in PneumaCult™-ALI-S Medium or PneumaCult™-ALI Medium at Passage 3, after 28 days. The ALI cultures were fixed and stained with antibodies for ciliated cells (AC-tubulin; green), club cells (SCGB1A1; magenta), and secretory protein (SCGB3A2; red). The nuclei were counterstained with DAPI (blue). Small airway markers, SCGB1A1 and SCGB3A2, were detected at higher levels in HSAECs cultured in PneumaCult™-ALI-S Medium compared with HSAECs cultured in PneumaCult™-ALI Medium and HBECs cultured in either PneumaCult™-ALI-S Medium or PneumaCult™-ALI Medium. All images were taken using a 63X objective. Scale bar = 50 µm.

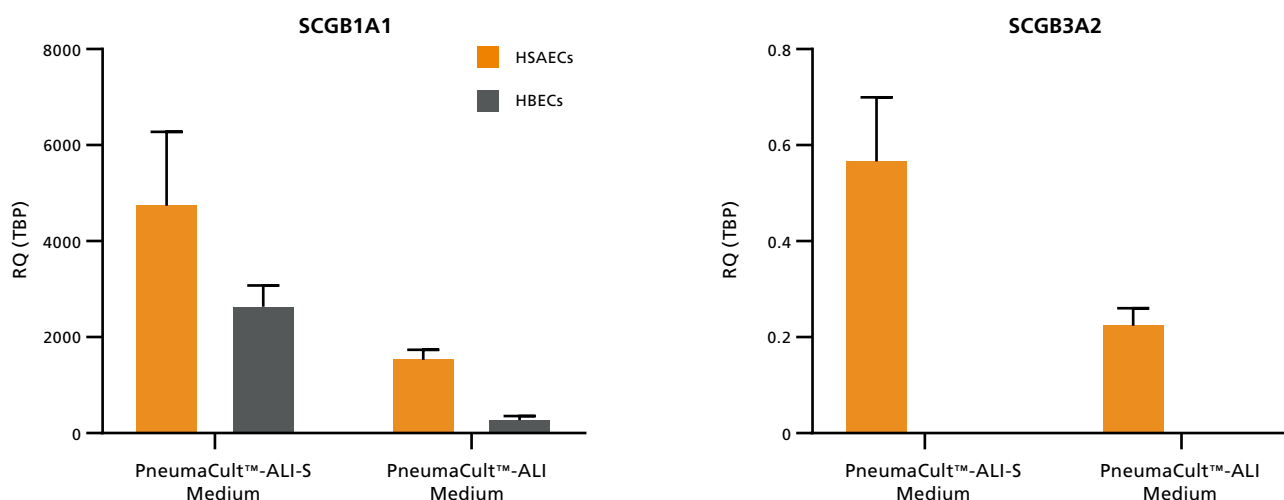
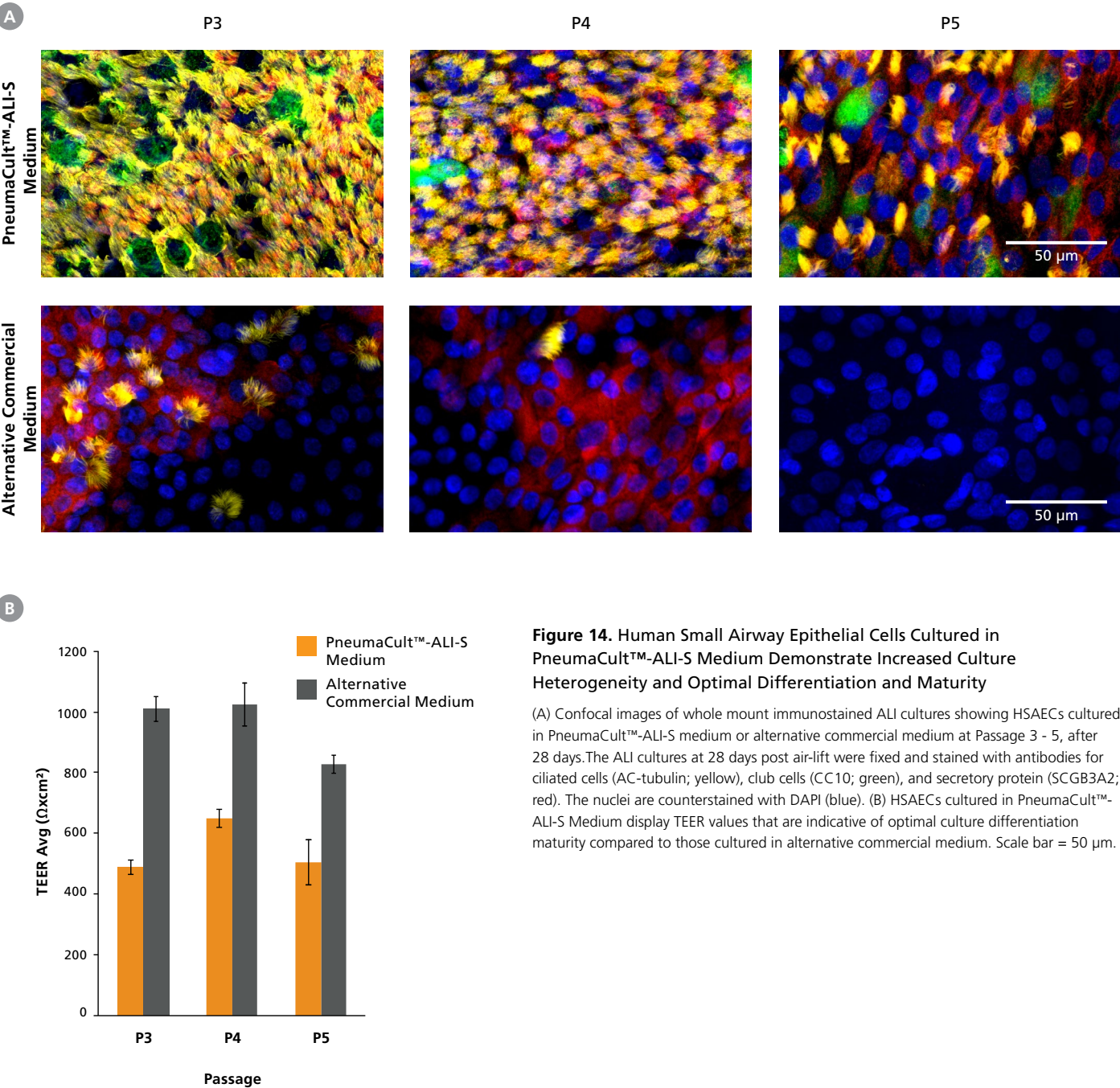


Figure 13. Human Small Airway Epithelial Cells Cultured in PneumaCult™-ALI-S Medium Express High Levels of Small Airway Epithelium Markers

HSAECs and HBECs expanded in PneumaCult™-Ex Plus Medium and differentiated in PneumaCult™-ALI-S Medium or PneumaCult™-ALI Medium at Passage 3. After 28 days of differentiation, the ALI cultures were analyzed for small airway epithelium markers (A) SCGB1A1 and (B) SCGB3A2. Gene-of-interest expression was normalized to housekeeping gene TBP and expressed as relative quantity (RQ). Relative expression of SCGB1A1 and SCGB3A2 was higher in HSAECs cultured in PneumaCult™-ALI-S Medium compared with HSAECs cultured in PneumaCult™-ALI Medium and HBECs cultured in either PneumaCult™-ALI-S Medium or PneumaCult™-ALI Medium. Relative expression of SCGB3A2 was not detectable in HBECs cultured in either PneumaCult™-ALI Medium or PneumaCult™-ALI-S Medium.

How Does PneumaCult™-ALI-S Medium Compare to Other Commercially Available Differentiation Media?

The differentiation performance of ALI cultures of HSAECs generated using PneumaCult™-ALI-S Medium or an alternative commercial medium were compared. HSAECs cultured in PneumaCult™-ALI-S Medium exhibited increased cellular heterogeneity (Figure 14A), as well as TEER values that are indicative of optimal culture differentiation maturity (Figure 14B).



Cell Culture Inserts

Cell culture inserts are an essential component of ALI cultures, and the quality and type of cell culture inserts used can greatly affect the performance of ALI cultures. STEMCELL offers multiple types of high-quality inserts that have been validated for use with PneumaCult™-ALI Medium.

Transwell® Inserts

Transwell® Inserts are recommended for culturing airway epithelial cells at the ALI. In a side-by-side comparison between Transwell® inserts and alternative commercially available inserts of the same material and pore size, ALI cultures generated by differentiating primary human bronchial epithelial cells (HBECs) in PneumaCult™-ALI Medium using Transwell® Inserts were more differentiated (Figure 15). qPCR analysis showed a higher expression of goblet and ciliated cell markers in the ALI cultures generated with Transwell® Inserts (Figure 16).

Why Use PneumaCult™-ALI Medium with Transwell® Inserts?

VALIDATED. Generate superior ALI culture morphology and epithelial cell marker expression with a validated protocol.

COMPATIBLE. Model the human airway at the ALI with inserts validated for use with PneumaCult™.

COMPLETE. Get all the tools to model the human airway at the ALI by using with PneumaCult™-Ex Plus and PneumaCult™-ALI Medium.

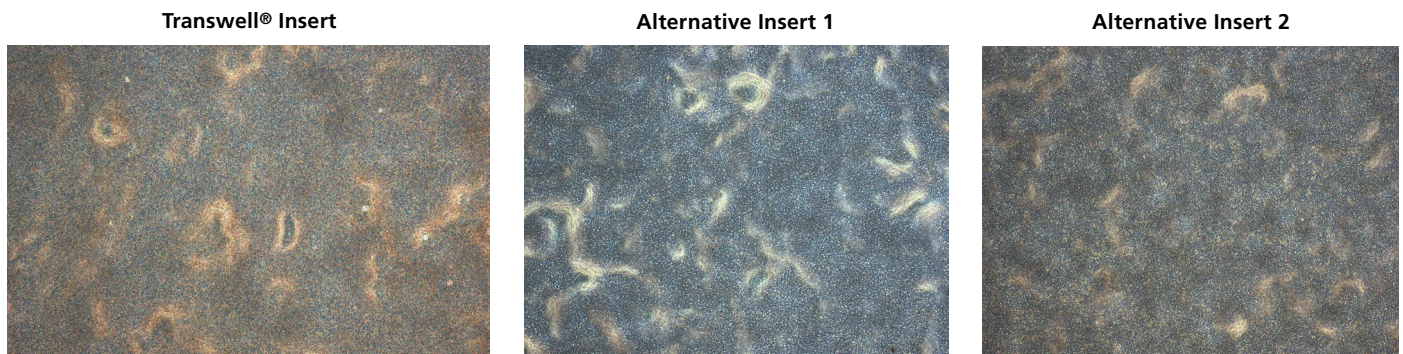


Figure 15. Air-Liquid Cultures of Human Bronchial Epithelial Cells Generated Using Transwell® Inserts Are More Differentiated

HBECs expanded in PneumaCult™-Ex Medium were seeded onto Transwell® inserts at Passage 3 and differentiated in PneumaCult™-ALI Medium for 21 days. Comparison was made with alternative commercially available inserts of the same material and pore size.

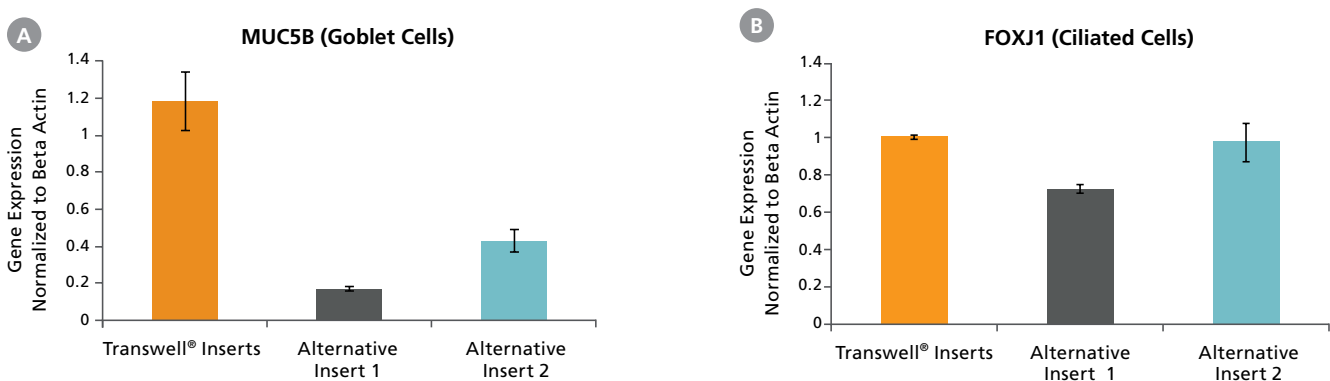


Figure 16. Air-Liquid Cultures of Human Bronchial Epithelial Cells Generated Using Transwell® Inserts Show Higher Differential Epithelial Cell Marker Expression

HBECs expanded in PneumaCult™-Ex Medium were seeded onto Transwell® inserts at Passage 3 and differentiated in PneumaCult™-ALI Medium for 21 days. Gene expression of (A) goblet (MUC5B) and (B) ciliated (FOXJ1) cell markers was assessed by qPCR and normalized to beta actin. Comparison was made with alternative commercially available inserts of the same material and pore size.

CELLTREAT® Inserts

CELLTREAT® Polyethylene Membrane Inserts support the growth of both anchorage-dependent and anchorage-independent cells, including those that are fed at the basolateral surface or in co-culture systems. These permeable inserts are recommended for culturing airway epithelial cells at the air-liquid interface; they can be used in studies related to infectious and genetic disease modeling, drug discovery, toxicity testing, and epithelial cell biology. ALI cultures generated using CELLTREAT® inserts produce functional and physiologically relevant pseudostratified columnar epithelium ready for downstream assays.

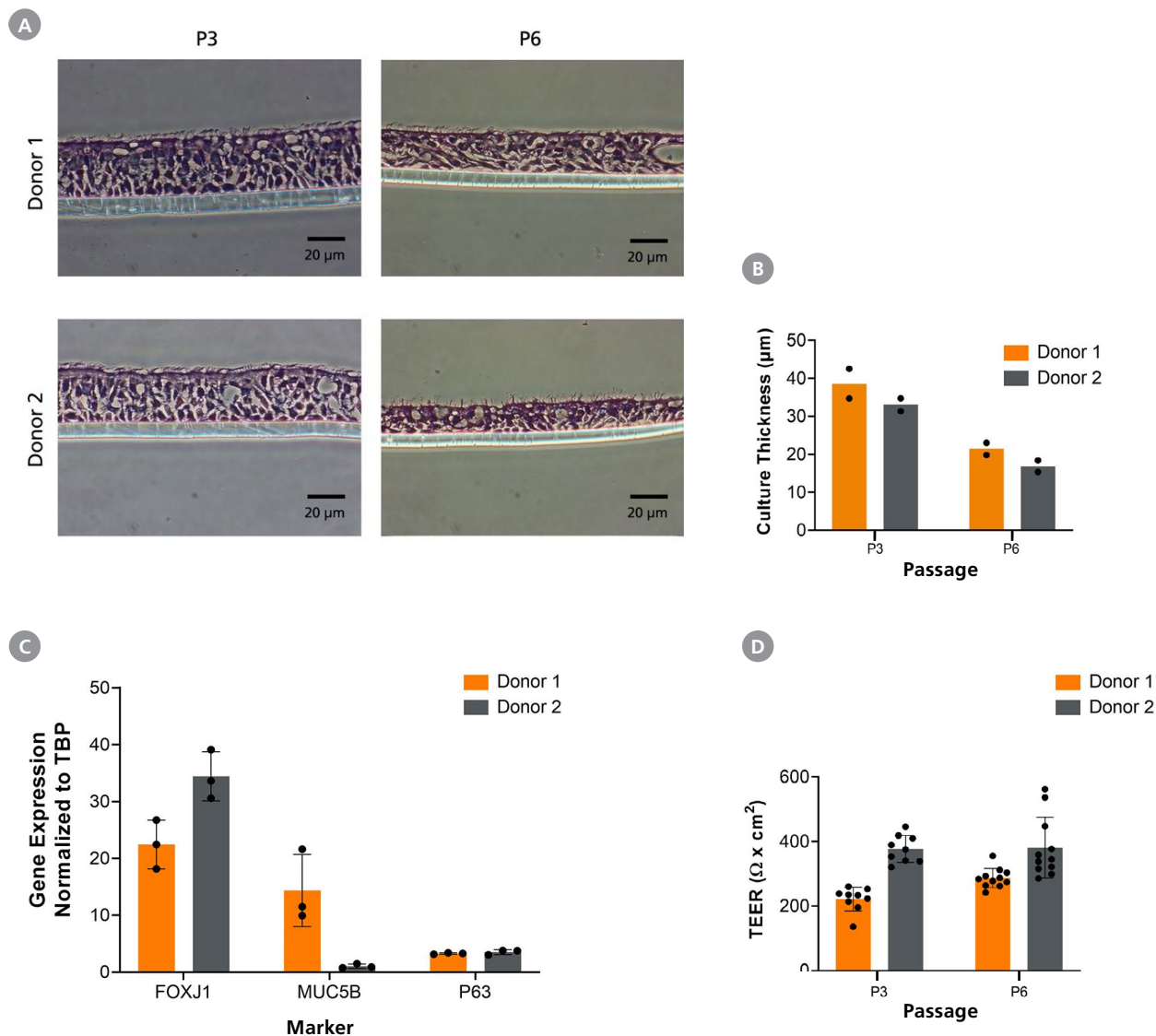


Figure 17. ALI Cultures Generated Using CELLTREAT® Inserts Form Pseudostratified Columnar Epithelium with High Expression of Large Airway Markers and Optimal Barrier Function

ALI cultures differentiated from HBECs at an early (P3) or late passage (P6) were fixed 4 weeks after air-lift. They were then paraffin embedded, sectioned, and stained with hematoxylin and eosin (H&E). Cultures generated using CELLTREAT® Inserts resulted in a pseudostratified columnar epithelium with visible cilia at the apical surface. An expected reduction in ALI culture thickness is observed with increasing HBEC passage number. (A) Representative H&E stained cross-sections; (B) ALI culture thickness (n = 2 replicate wells from a single experiment). (C) Expression of large airway markers for ciliated (FOXJ1), goblet (MUC5B), and basal (P63) cells were assessed by qPCR and normalized to the housekeeping gene TBP. Error bars represent standard deviation (n = 3 replicate wells from a single experiment). (D) TEER measurements of ALI cultures differentiated from HBECs at an early (P3) or late passage (P6) were taken 4 weeks after air-lift. Values were corrected against blank wells. Average values of donors were within the expected normal physiological levels (200 - 800 Ω x cm²), indicative of optimal culture differentiation. Error bars represent standard deviation (n ≥ 9 replicate wells from a single experiment).

Differentiation of Human Primary Airway Epithelial Cells as Airway Organoids

Airway Organoid Culture Systems

Airway organoid culture systems are *in vitro* models that mimic the structure and function of the large and small airways of the respiratory system. These models are derived from human primary bronchial or airway epithelial cells to generate three-dimensional cultures that provide a more accurate representation of the *in vivo* tissue when compared to two-dimensional cell culture. With PneumaCult™ Airway Organoid culture systems, organoids can be differentiated with easy access to the basal or apical side according to research needs.

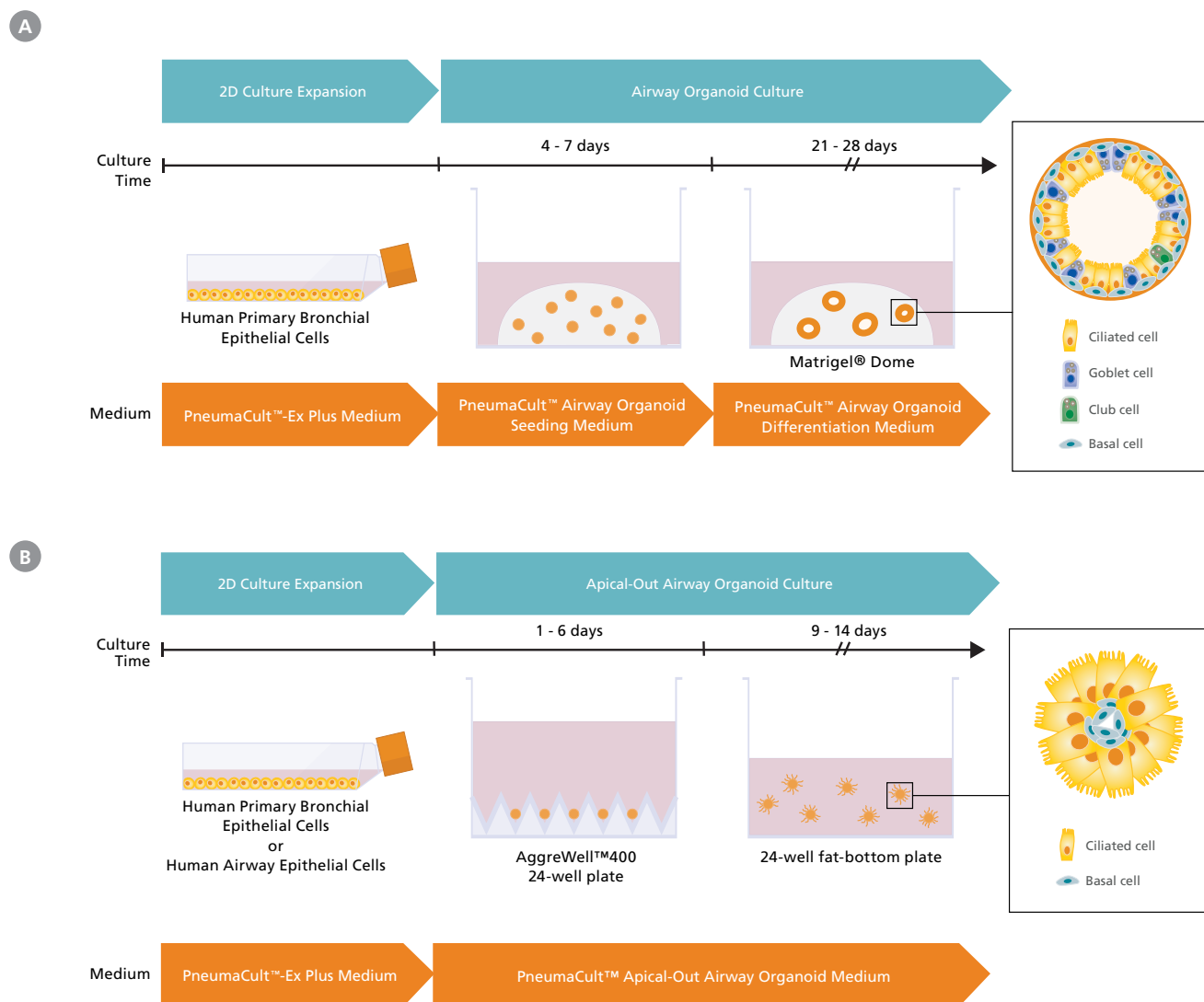


Figure 18. Overview of the PneumaCult™ Airway Organoid Culture Systems

(A) In the early two-dimensional expansion phase of the human airway organoid culture procedure, HBECs are expanded using PneumaCult™-Ex Plus Medium. The HBECs are then embedded into a Matrigel® dome and expanded for 4 - 7 days using PneumaCult™ Airway Organoid Seeding Medium. Following the expansion, the HBECs are differentiated using PneumaCult™ Airway Organoid Differentiation Medium for an additional 21+ days. (B) In the two-dimensional expansion phase of the human apical-out airway organoid culture protocol, HBECs or HAECS are expanded using PneumaCult™-Ex Plus Medium. The HBECs or HAECS are then plated into an AggreWell™400 24-well plate and allowed to aggregate for 1 - 6 days using PneumaCult™ Apical-Out Airway Organoid Medium. Following the aggregation, the cells are dislodged from the microwells and the aggregate suspension is transferred to a 24-well flat-bottom plate and differentiated for 9 - 14 days using PneumaCult™ Apical-Out Airway Organoid Medium to generate apical-out airway organoids that display beating cilia.

PneumaCult™ Airway Organoid Kit

PneumaCult™ Airway Organoid Kit is a novel serum-free medium kit that supports the efficient generation of fully differentiated and functional airway organoids from both healthy and disease samples. Airway organoid cultures provide an alternative method to ALI-based cultures for in vitro human airway modeling. Since this culture system does not require the use of cell culture inserts, it is amenable to high-throughput drug screening and can be used in large-scale screening for CFTR modulators. Fully differentiated human airway organoids recapitulate key features of the in vivo human airway, such as a hollow lumen surrounded by a polarized airway epithelial cell layer consisting of ciliated cells and goblet cells.

PneumaCult™ Airway Organoid Kit provides the necessary components to prepare PneumaCult™ Airway Organoid Seeding Medium, which allows for initiation of 3D organoid culture, and PneumaCult™ Airway Organoid Differentiation Medium, to further obtain morphologically representative and fully differentiated human airway organoids (Figure 19).

Why Use PneumaCult™ Airway Organoid Kit?

PHYSIOLOGICAL. Recapitulate the in vivo human airway using a three-dimensional in vitro system.

COMPLETE. Expand and differentiate human airway epithelial cells to airway organoids by using with PneumaCult™-Ex Plus Medium.

RELIABLE. Maximize experimental reproducibility with rigorous raw material screening and extensive quality control testing.

USER-FRIENDLY. Get started now with a convenient, cell culture insert-free format and easy-to-use protocol.

Morphological and Functional Characterization of Fully Differentiated Human Airway Organoids

Commercially available HAEs at Passage 1 from both healthy and cystic fibrosis (CF) donors were expanded and serially passaged using PneumaCult™-Ex Plus Medium. At passages 2 to 5, 2×10^3 HAEs were embedded into a 50 μ L Matrigel® dome using PneumaCult™ Airway Organoid Seeding Medium (Figure 19A). After 7 days of expansion, the medium was switched to PneumaCult™ Airway Organoid Differentiation Medium (Figure 19B). Following differentiation, the organoids demonstrated lumens and robust cilia beating inside the lumen (Figure 19C).

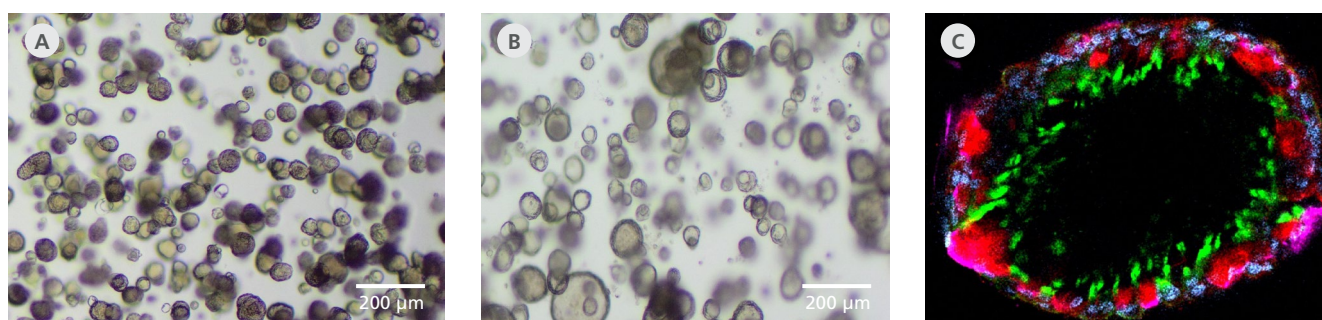


Figure 19. Fully Differentiated Human Airway Organoids Can Be Generated Using PneumaCult™ Airway Organoid Kit

(A) Bright-field image of airway organoids growing in PneumaCult™ Airway Organoid Seeding Medium at Day 7 demonstrating basal cell spheroid morphology. (B) Bright-field image of airway organoids differentiated in PneumaCult™ Airway Organoid Differentiation Medium at Day 21 exhibiting hollow lumen. (C) Airway organoid was stained for ZO-1 (junction protein marker, red), MUC5AC (goblet cell marker, purple), AC-Tubulin (ciliated cell marker, green), and DAPI (nuclei, blue). Scale bar = (A-B) 200 μ m and (C) 25 μ m.



RESOURCE

Learn About Lung Organoid Cultures
www.stemcell.com/Lung-Organoids

Should you intend to use this product for commercial purposes, please contact HUB at www.huborganoids.nl for a commercial use license or for clarifications in relation to HUB licensing.

To assess their suitability for CFTR assays, fully differentiated airway organoids grown from either healthy or CF donors using the PneumaCult™ Airway Organoid Kit, were treated with either dimethyl sulfoxide (DMSO) control or 20 μ M amiloride, 10 μ M forskolin, and 25 μ M genistein, and allowed to incubate for 6 hours to bring about forskolin-induced swelling. The surface area of the organoids was measured (Figure 20A and 20B). Organoids were also collected in cold Corning® Cell Recovery Solution. Following their enzymatic dissociation in ACCUTASE™ into a single-cell suspension, the organoids were quantified for the number of ciliated cells. The ciliated cell percentage in organoids grown from both healthy (Figure 21A) and CF donors (Figure 21B) increased from Passage 3 - 5.

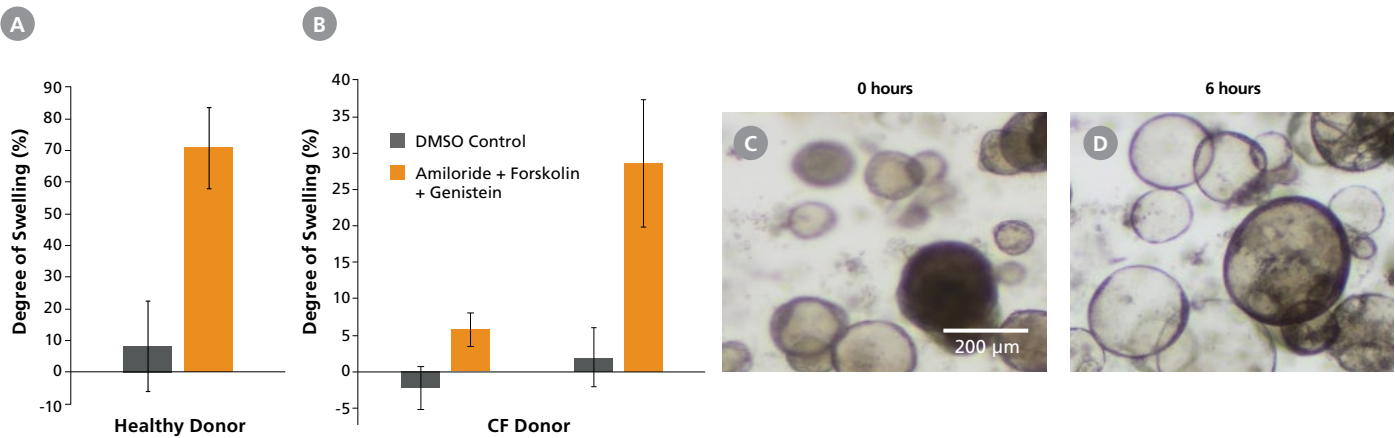


Figure 20. Airway Organoids Are Suitable for Assessing CFTR Protein Expression Using Forskolin-Induced Swelling Assay

(A) Forskolin-treated organoids derived from healthy donors increased in size compared to the DMSO control, indicating functional CFTR protein expression. (B) Forskolin-induced swelling is lost in organoids derived from CF donors, but re-established in VX-809-treated airway organoids. Error bars represent \pm 95% confidence interval for the mean (n=3). Bright-field images of airway organoids taken during the Forskolin swelling assay at (C) 0 hours and (D) 6 hours show organoid swelling after treatment. Scale bar = 200 μ m.

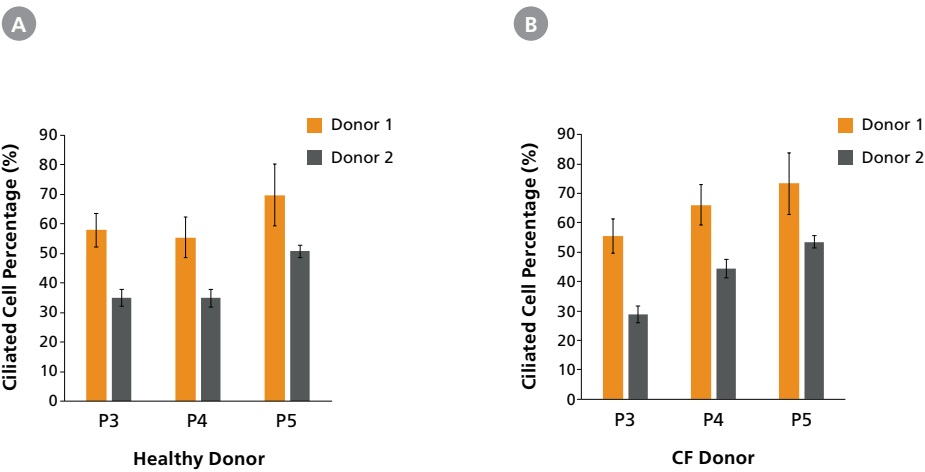


Figure 21. Fully Differentiated Airway Organoids Retain Morphological Characteristics at Different Passages

The ciliated cell percentage in organoids grown from (A) healthy and (B) CF donors using PneumaCult™ Airway Organoid Kit increased from Passage 3 to 5. The total number of cells and the number of ciliated cells were counted using a hemocytometer. Error bars represent \pm 95% confidence interval for the mean (n=3).

PneumaCult™ Apical-Out Airway Organoid Medium

PneumaCult™ Apical-Out Airway Organoid Medium is a serum- and BPE-free cell culture medium that supports the highly reproducible generation of apical-out airway organoids in 15 days from human bronchial epithelial cells (HBECs) or human airway epithelial cells (HAECs). These polarized airway organoids (with outward-facing, differentiated ciliated cells) provide access to the apical side of the airway epithelium and can be used to perform infectious disease modeling or high-throughput drug screening. For a complete apical-out airway organoid culture workflow, HBECs or HAECs can be first expanded in PneumaCult™-Ex Plus Medium prior to initiating organoid culture.

Why Use PneumaCult™ Apical-Out Airway Organoid Medium?

CONVENIENT. Easily access the apical side of the airway epithelium.

MATRIX-FREE. Perform simple downstream processing with a Matrigel®-free protocol.

CONSISTENT. Generate homogeneous organoids across donors.

USER-FRIENDLY. Get started now with a convenient, cell culture insert-free format and easy-to-use protocol.

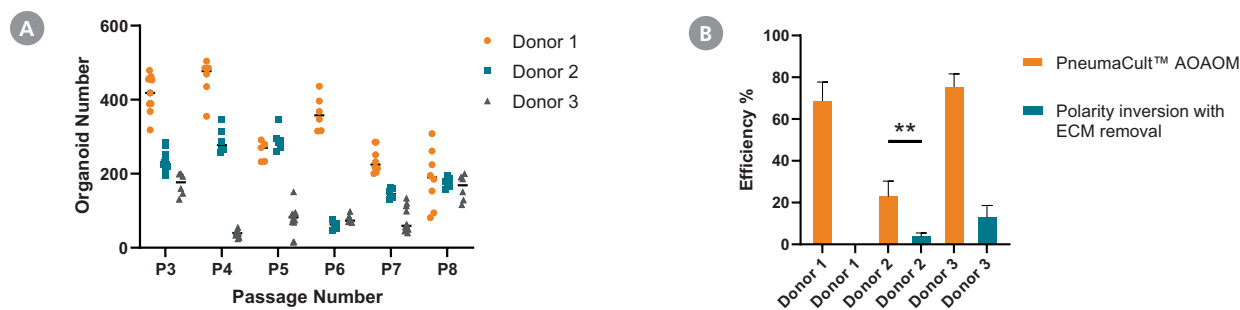


Figure 22. PneumaCult™ Apical-Out Airway Organoid Medium Supports Efficient Generation of Organoids

Apical-out airway organoids were generated by seeding 100 2D-expanded cells derived from 3 donors from passages 3 to 8. (A) Number of generated organoids per well of a 24-well plate at Day 15. Data points represent measurements taken from independent wells of a 24-well plate. (B) The efficiency of two different approaches (ECM-free PneumaCult™ Apical-Out Airway Organoid Medium workflow or polarity inversion following ECM removal) is expressed as the percentage of apical-out airway organoids at the end of culture relative to the total number of organoids used to seed the cultures.

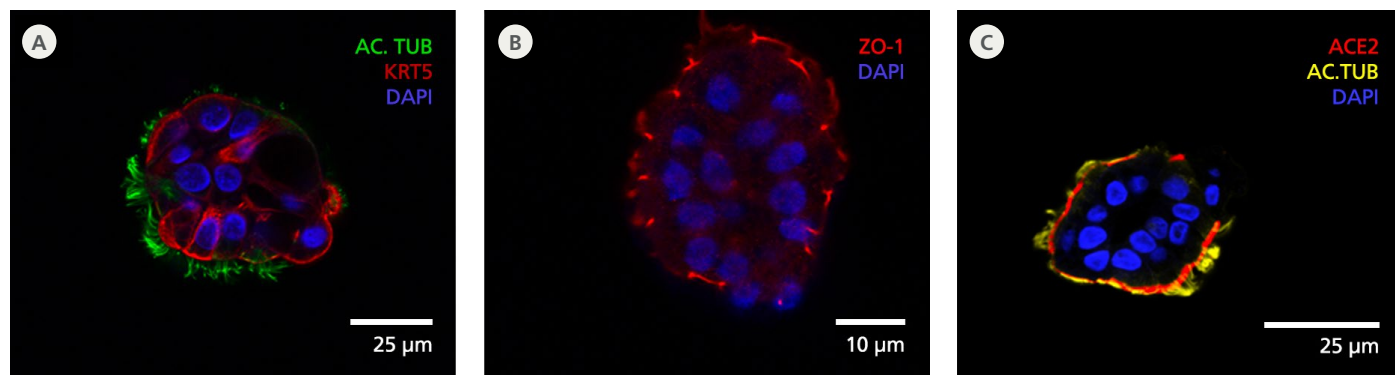


Figure 23. Terminally Differentiated Organoids Exhibit Mature Polarized Airway Epithelium

(A) Day 15 apical-out airway organoids cultured in PneumaCult™ Apical-Out Airway Organoid Medium contain ciliated cells, as confirmed by the presence of acetylated tubulin (AC. TUB; green) on the outward-facing apical cell surface, while keratin 5 (KRT5; red)-expressing basal cells were present alongside ciliated cells. (B) Cell-cell tight junction protein ZO-1 (red) can be readily visualized on the apical side of the organoid. These results indicate efficient generation of organoids across multiple passages with successful apical-out orientation. (C) Presence of ciliated cell marker acetylated tubulin (AC.TUB; yellow) and SARS-CoV-2 key entry marker ACE2 (red) is also shown, suggesting their usefulness for modeling respiratory infection from SARS-CoV-2.

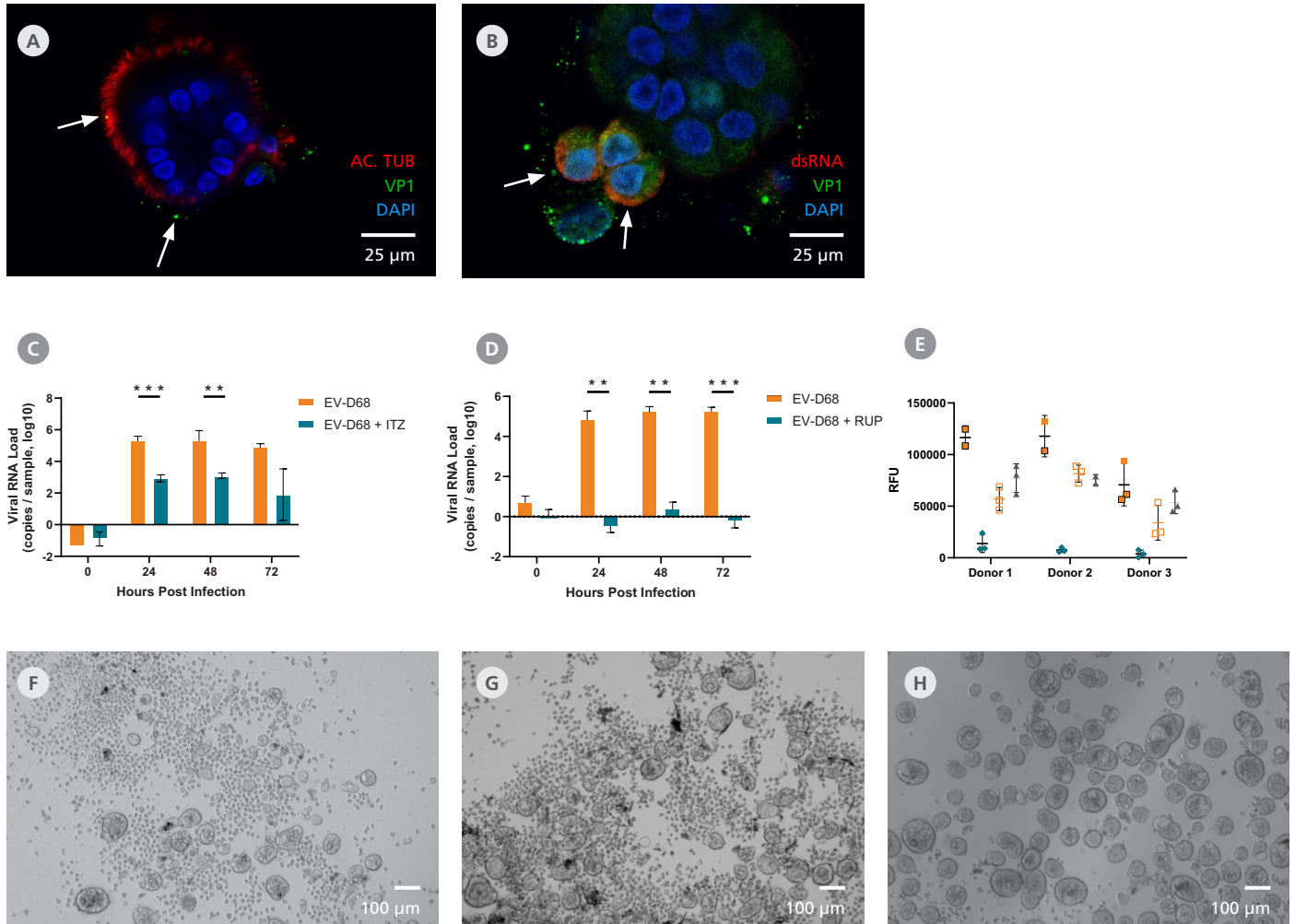


Figure 24. Mature Organoids Were Incubated with Enterovirus-D68 in the Presence or Absence of Itraconazole (ITZ) or Rupintrivir (RUP) to Study Antiviral Effects

To assess whether the PneumaCult™ Apical-Out Airway Organoid system was susceptible to infection to common respiratory viruses, Day 15 differentiated organoids were infected with enterovirus-D68 (EV-D68). (A) At 0 hours post infection, the EV-D68 (VP1; green) could be seen binding to the apical surface (ciliated cells; acetylated tubulin; red). (B) After 6 hours, cells containing double-stranded RNA (dsRNA; red), an intermediate stage during the viral replication cycle, can be identified. These cells are also positive for viral protein (VP1; green) and (C and D; orange bars) generate high viral RNA titers, (F) while showing evidence of cytopathogenic effect (CPE). (C; teal bars) Treatment with Itraconazole (ITZ) reduced the viral RNA levels by approximately 2 orders of magnitude and (G) slightly ameliorated the CPE levels. (D; teal bars) Treatment with Rupintrivir (RUP) completely inhibited viral replication, (H) resulting in the absence of any CPE. (E) Measuring the relative fluorescent units (RFU) 72 hours post infection indicated changes in the levels of ATP and viability of the organoids and revealed a sharp decline in viability following infection with EV-D68. A partial rescue was detected in infected apical-out airway organoids treated with ITZ, whereas those treated with RUP were almost completely rescued. Double asterisks indicate $p < 0.01$ and three asterisks indicate $p < 0.001$ ($n=3$).

Culturing Alveolar Organoids

Modeling the Alveolar Region of the Lung

The alveoli are the respiratory units of the lung and are located in the most distal portion of the airway. The alveolar sacs are composed of alveolar type 1 (AT1) and type 2 (ATII) epithelial cells that are responsible for gas exchange and self-renewal, respectively. Traditional two-dimensional (2D) culture techniques have proven to be insufficient for long-term culture of ATII cells due to the lack of optimized culture conditions to maintain their ATII cell phenotype. By using alveolar organoids, the regional specificity of the in vivo human alveoli is captured, making them ideally suited for alveolar biology research, infectious disease studies, and drug screening.

PneumaCult™ Alveolar Organoid Media can be used to obtain mature and fully differentiated organoids that recapitulate key features of in vivo ATII and AT1 cells.

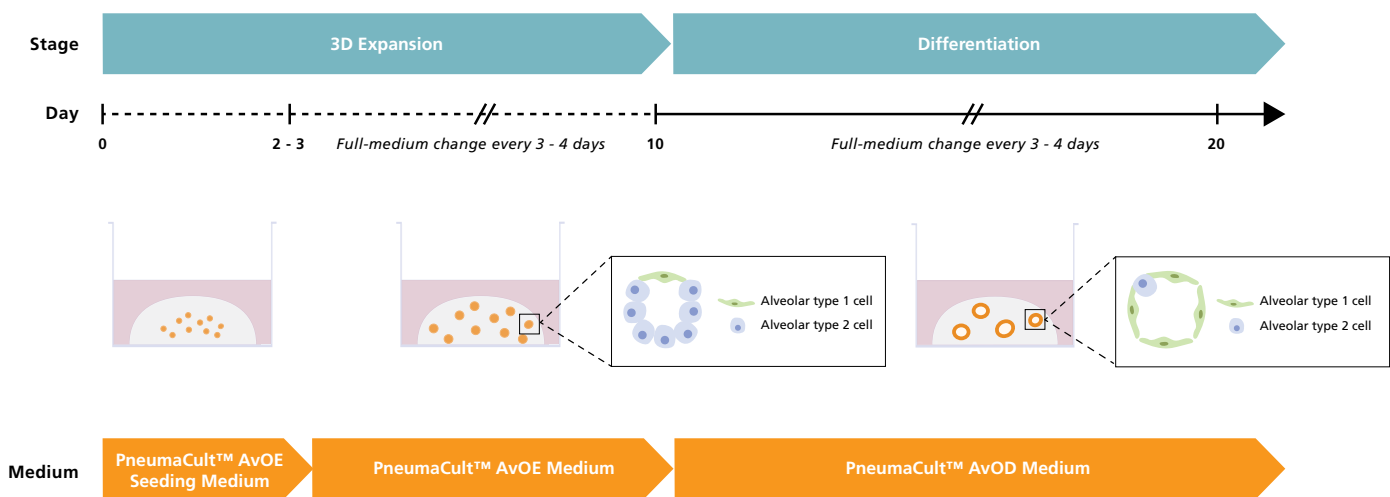


Figure 25. Overview of PneumaCult™ Alveolar Organoid System

The PneumaCult™ Alveolar Organoid Media workflow is a two-phase protocol. During the expansion phase, primary isolated or cryopreserved human ATII single cells are seeded in a Matrigel® dome and cultured with PneumaCult™ Alveolar Organoid Expansion (AvOE) Seeding Medium. On Day 2 - 3, after a full-medium change, cultures are expanded using PneumaCult™ AvOE Medium to obtain mature ATII organoids. In the differentiation phase, ATII organoids are cultured for 10 additional days using PneumaCult™ Alveolar Organoid Differentiation (AvOD) Medium to generate AT1 organoids.

PneumaCult™ Alveolar Organoid Media

PneumaCult™ Alveolar Organoid Expansion (AvOE) Medium enables the passage and expansion of human alveolar type 2 (ATII) epithelial cells long term as organoids. These alveolar organoids maintain properties indicative of the ATII cell phenotype, including the ability for self-renewal, expected marker expression (HT2-280 and SP-C), and lineage potential for differentiation to alveolar type I (ATI) epithelial cells. PneumaCult™ Alveolar Organoid Differentiation (AvOD) Medium can be used to differentiate the expanded ATII organoids to ATI organoid cultures in as few as 10 days. Differentiated cells show decreased ATII marker expression and strong up-regulation of ATI cell markers (RAGE/AGER, HT1-56, and GPRC5a).

PneumaCult™ AvOE and AvOD Media are compatible with primary isolated fresh or cryopreserved ATII cells, as well as high-quality commercially available alveolar epithelial cell sources.

Why Use PneumaCult™ Alveolar Organoid Media?

RELEVANT. Model the human alveolar physiology in 3D with an in vitro culture system that recapitulates key features of ATII and ATI cells in vivo.

BIOBANK. Cryopreserve and re-initiate organoid cultures with the biobanking capabilities of the media.

HIGH-YIELD. Maximize yield from the initial sample with a medium that supports passaging and long-term expansion of ATII organoids.

SIMPLE. Generate mature ATII and fully differentiated ATI organoids with a convenient format and easy-to-use protocol

RELIABLE. Gain confidence in your results with standardized media and rigorous quality control testing.

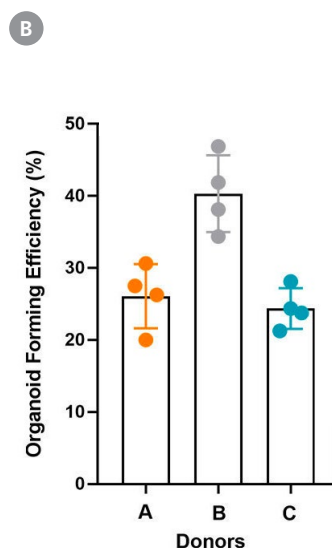
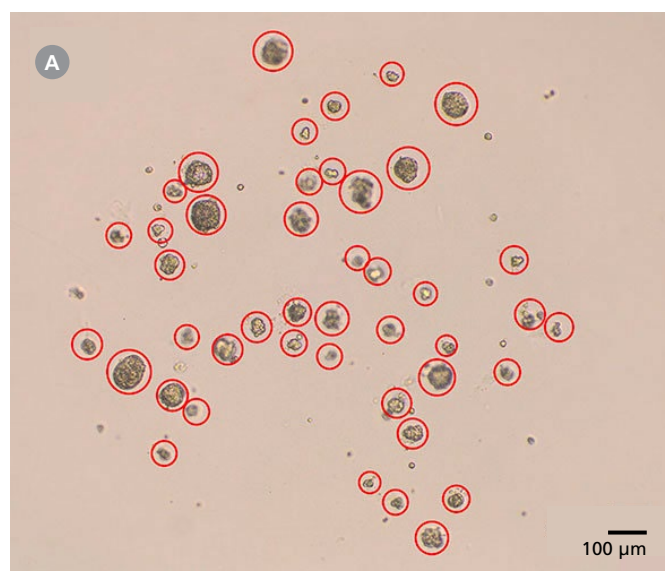


Figure 26. PneumaCult™ Alveolar Organoid Expansion Medium Supports High Organoid Forming Efficiency

Organoid forming efficiency (OFE) serves as a functional measure of the health of an organoid culture and the performance of the media. ATII organoids were expanded in PneumaCult™ AvOE Medium (160 cells seeded per dome). (A) Brightfield image taken at Day 9 using a 4X objective. The entire dome was captured in the image, and each organoid formed was counted and indicated (total 44; OFE = 27.5%). (B) Bar graph showing OFE of ATII organoid cultures derived from three different donors (means \pm SD). OFE = (organoids formed/cells seeded) \times 100%.



WEBINAR

Modeling the Alveolar Region with Organoids
www.stemcell.com/Alveolar-Webinar

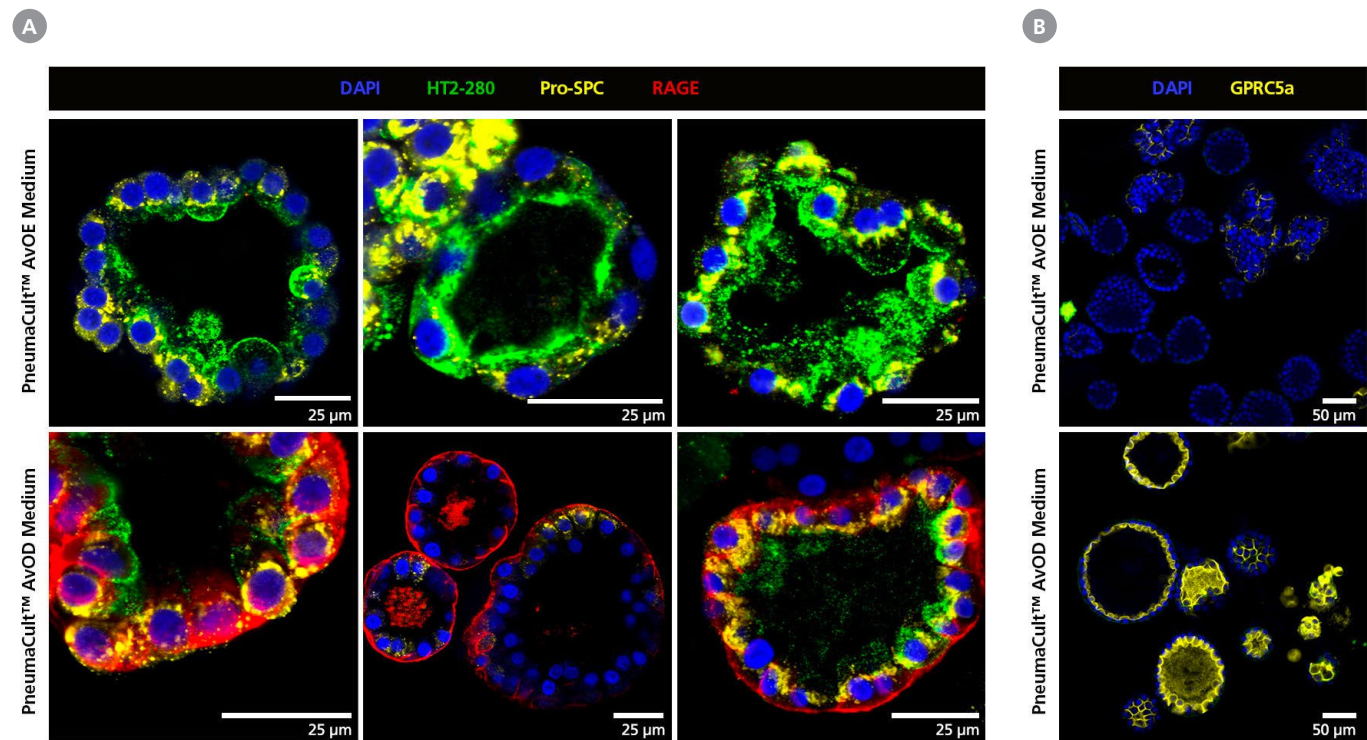


Figure 27. Immunohistochemistry Confirms Expression of ATII and ATI Cell Markers in Alveolar Organoids
(A) Organoids from three donors expanded in PneumaCult™ AvOE Medium (top) express the ATII markers, HT2-280 (green) and pro-surfactant protein C (pro-SPC, yellow). When further differentiated in PneumaCult™ AvOD Medium (bottom), the levels of these ATII markers are down-regulated, while the ATI marker RAGE (red) is upregulated. (B) Differentiated organoids also express high levels of ATI marker GPRC5a (yellow).

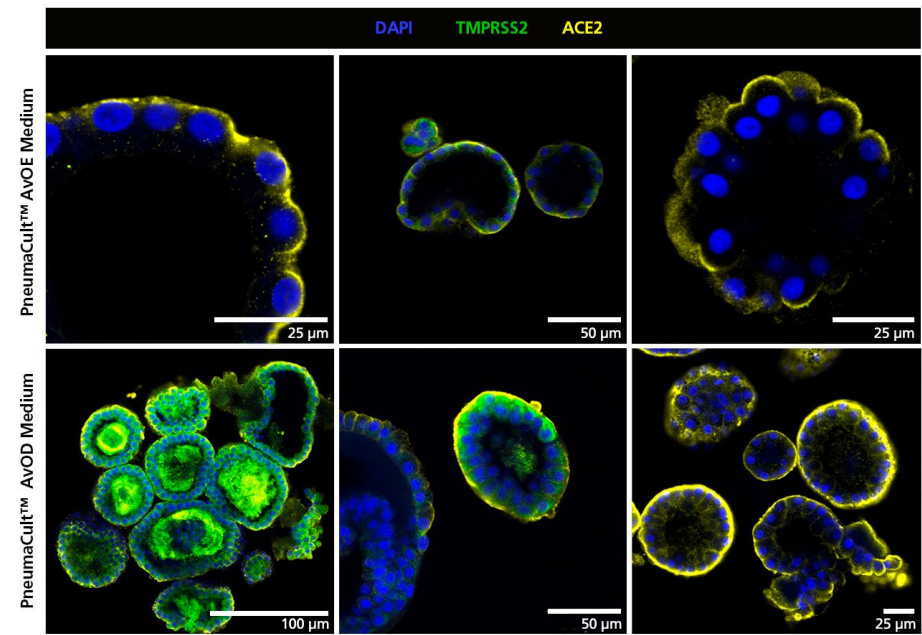


Figure 28. Organoids Cultured in PneumaCult™ Alveolar Organoid Media Express TMPRSS2 and ACE2, Key Markers for SARS-CoV-2 Entry
Organoids from three donors expanded in PneumaCult™ AvOE Medium (top) and differentiated in PneumaCult™ AvOD Medium (bottom) express proteins associated with SARS-CoV-2 entry, TMPRSS2 and ACE2. While ACE2 was expressed in all donors and conditions, TMPRSS2 expression was donor-dependent.

Protocol: How to Culture Alveolar Monolayers from ATII Organoids

Having the right alveolar model is crucial for providing important insights on applications in the alveolar space. In combination with STEMCELL's PneumaCult™ Alveolar Organoid System, this two-stage protocol describes the generation of alveolar monolayers from expansion-phase alveolar type 2 (ATII) organoids to facilitate air-liquid interface (ALI) culture and enable easy access to the apical surface.

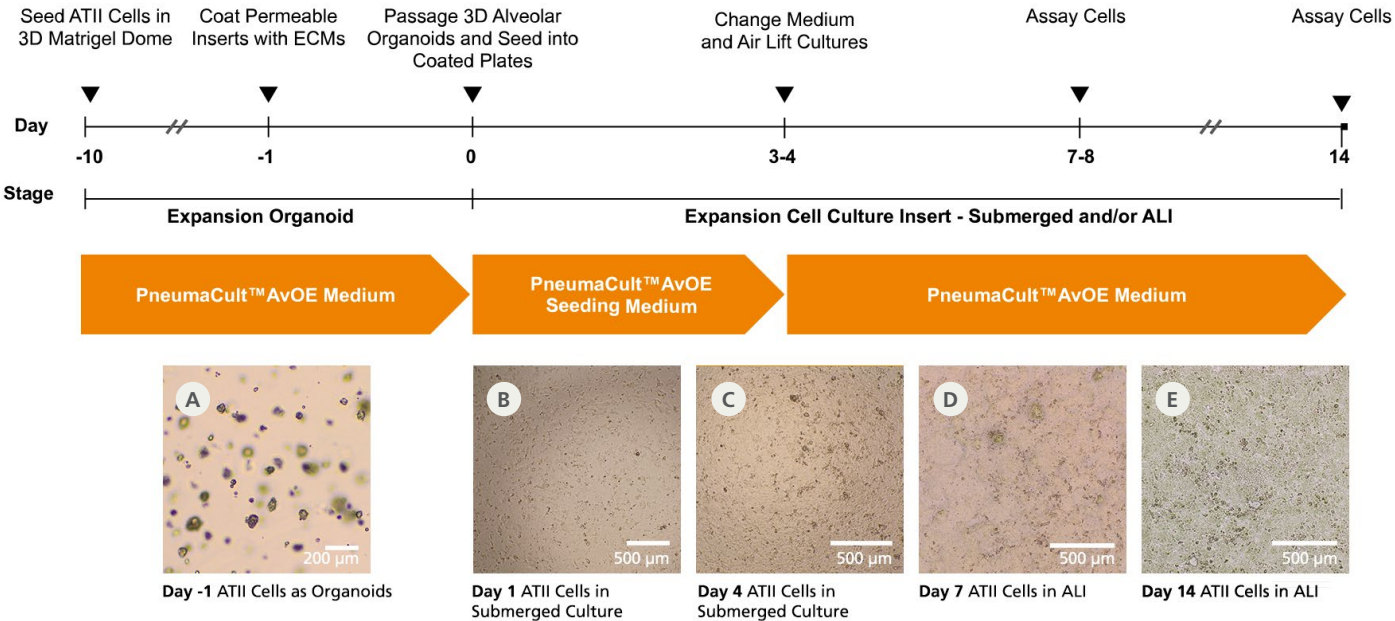


Figure 29. Generating ATII Cultures at the Air-Liquid Interface

The generation of ATII cultures at the ALI on cell culture inserts is a two-stage protocol. (A) In the first stage, primary isolated or cryopreserved human ATII single cells are seeded in PneumaCult™ Alveolar Organoid Expansion (AvOE) Medium as organoid cultures for 10 days. Prior to seeding on cell culture inserts, the inserts are coated with a combination of 2 extracellular matrices (ECMs). (B) Single cells are then harvested from the expanded ATII organoids and seeded into the extracellular matrix-coated cell culture inserts before being expanded for 3 - 4 days in PneumaCult™ Alveolar Organoid Seeding Medium. (C) After confluency is reached, the cells may be airlifted for ALI culture and (D) collected for assays on Day 7 or (E) cultured for up to an additional 10 days.

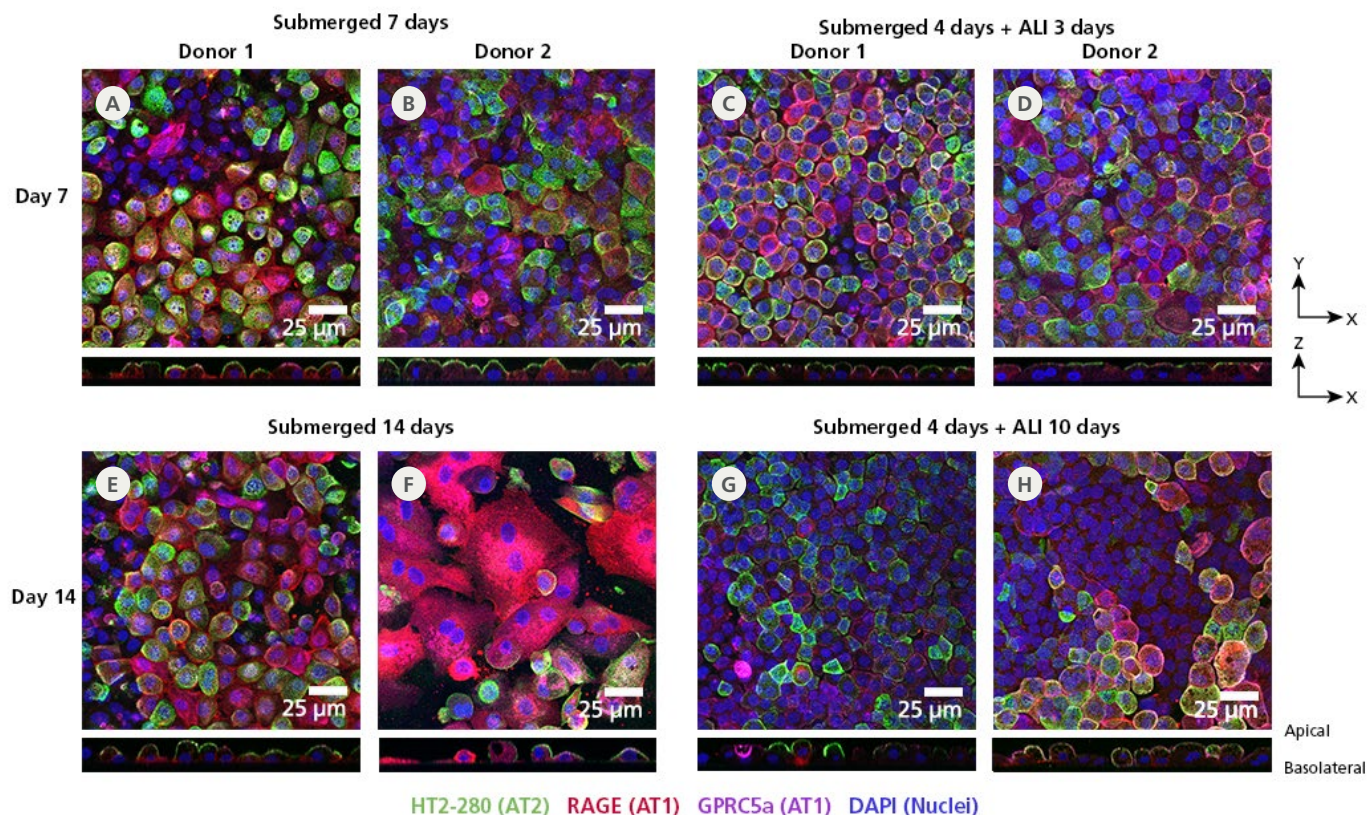


Figure 30. ATII Cells Show Optimal Marker Expression When Cultured for 7 Days As Submerged or ALI Cultures

ATII cells from two separate donors were seeded into ECM-coated inserts and cultured to Day 7 or Day 14. Cultures were placed at ALI on Day 4 and cultured for an additional (C, D) 3 or (G, H) 10 days. Cells were fixed on the membrane in 4% PFA and stained for the ATII-specific marker HT2-280 (green), as well as the ATI-specific markers RAGE (red) and GPRC5a (magenta) and counterstained with DAPI (blue). In both donors, cultures in (A, B) submerged and (C, D) ALI for 7 days express HT2-280 with some RAGE, and little GPRC5a. Additional growth for 7 days in (E, F) submerged cultures resulted in a decrease in HT2-280 and an increase in RAGE, indicating differentiation into ATI cells. (G, H) Growth for an additional 7 days at the ALI resulted in some decrease in HT2-280, but expression was more stable than in submerged culture conditions. This data suggests that the ideal culture conditions for ATII cells on cell culture inserts are for (C, D) 7 days, with 3 days at ALI; however these cultures can still be maintained for up to 14 days (10 days of ALI) with minimal loss of ATII markers. Note, scale is the same for XY and ZX figures.



PROTOCOL

How to Culture Alveolar Monolayers
www.stemcell.com/AT2-Protocol

Culturing hPSC-Derived Lung Organoids

STEMdiff™ Lung Progenitor Kit

The STEMdiff™ Lung Progenitor Kit is a serum-free culture medium system for efficient and reproducible generation of lung progenitor cells from human pluripotent stem cells (hPSCs), including embryonic stem (ES) and induced pluripotent stem (iPS) cells. Differentiated cells will express NKX2.1, a key marker of lung progenitor cells. The resulting cells can be further matured toward proximal or distal airway cells, using published protocols, for the study of lung diseases and lung development.

Why Use the STEMdiff™ Lung Progenitor Kit?

- REPRODUCIBLE.** Maximize experimental reproducibility with an optimized, serum-free formulation.
- RELEVANT.** Obtain lung progenitor cells capable of downstream differentiation to proximal and distal airway cells.
- CONVENIENT.** Follow an easy-to-use, 15-day protocol.

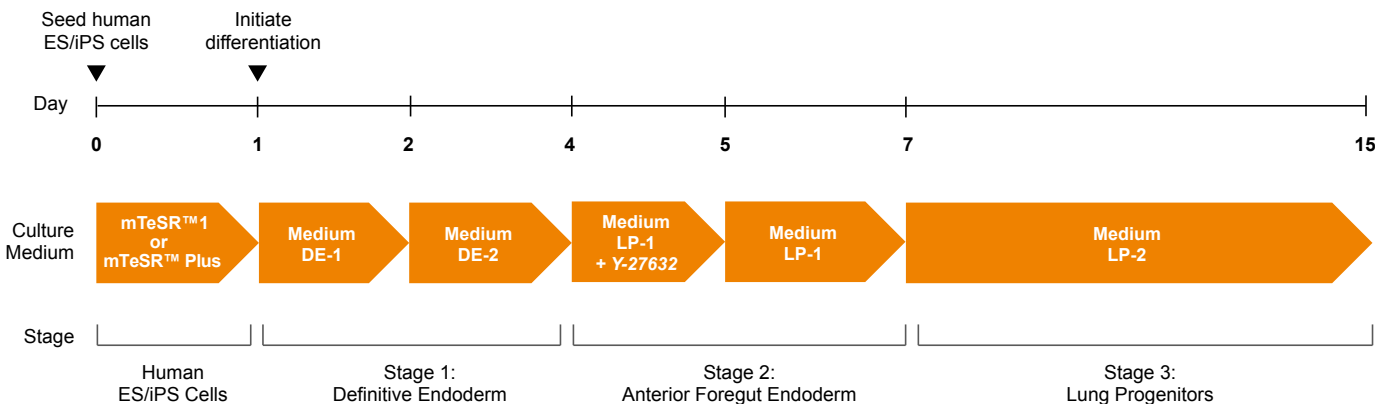


Figure 31. Schematic for Generating Lung Progenitor Cells from Human ES/iPS Cells Using STEMdiff™ Lung Progenitor Kit

hPSC cultures progress through a simple three-stage process to generate lung progenitor cells. hPSC clumps are first seeded in mTeSR™1 or mTeSR™ Plus. On Day 1, differentiation is initiated with Medium DE-1 (STEMdiff™ Endoderm Basal Medium containing Supplement MR and Supplement CJ). Subsequently on Day 2 and 3, the medium is changed to Medium DE-2 (STEMdiff™ Endoderm Basal Medium containing Supplement CJ) for definitive endoderm patterning. On Day 4, to initiate anterior foregut endoderm patterning, the endoderm monolayer is passaged in Medium LP-1 (STEMdiff™ Lung Basal Medium, Lung Supplement (10X), and Supplement 1) and Y-27632. Finally, at Day 7, the cells are differentiated into the lung progenitor stage with Medium LP-2 (STEMdiff™ Lung Basal Medium, Lung Supplement (10X), and Supplement 2).



PRODUCT

Learn More About STEMdiff Lung Progenitor Kit
www.stemcell.com/STEMdiff-Lung-Progenitor

STEMdiff™ Branching Lung Organoid Kit

The STEMdiff™ Branching Lung Organoid Kit supports the efficient and reproducible generation of branching lung organoids from human ES and iPS cells through four stages of differentiation:

- Definitive endoderm
- Anterior foregut endoderm
- Lung bud organoid
- Branching lung organoids

This serum-free medium system generates organoids that develop proximal and distal-like branching airway epithelial structures, and can be matured for extended periods of organoid culture (> 28 days) using STEMdiff™ Branching Lung Organoid Maturation Kit.

Why Use STEMdiff™ Branching Lung Organoid Kit?

PHYSIOLOGICALLY RELEVANT. Generate organoids that recapitulate in vivo airway branching morphogenesis and proximo-distal specification.

ROBUST. Efficiently differentiate multiple human ES and iPS cell lines to branching lung organoids.

CONVENIENT. Get experimental flexibility with a cryopreservable intermediate stage.

REPRODUCIBLE. Maximize experimental reproducibility with a serum-free, defined formulation.

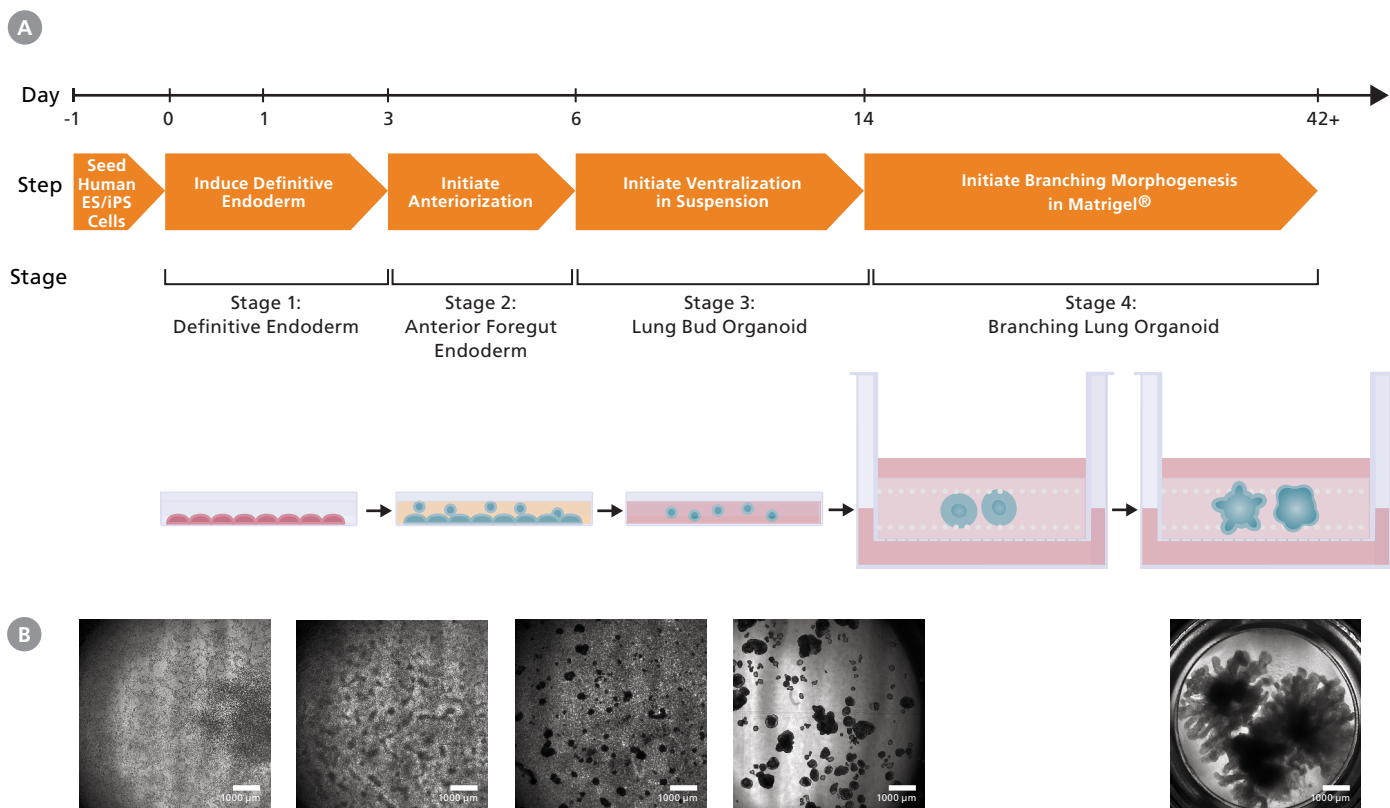


Figure 32. STEMdiff™ Branching Lung Organoid Kit Supports Generation of Branching Lung Organoids

(A) Human PSC cultures progress through a four-stage differentiation process to generate human branching lung organoids. By the end of stage 1 (Day 3), cultures exhibit characteristics typical of definitive endoderm and anterior foregut differentiation is initiated. During stage 2 (Days 3 - 6), anterior foregut endoderm buds are released from the monolayer, and are then suspended to form ventralized lung bud organoids in stage 3 (Days 6 - 14). In stage 4, the lung bud organoids are embedded into Matrigel® sandwich cultures to mature into branching lung organoids. (B) Morphological representation of the culture at different stages.

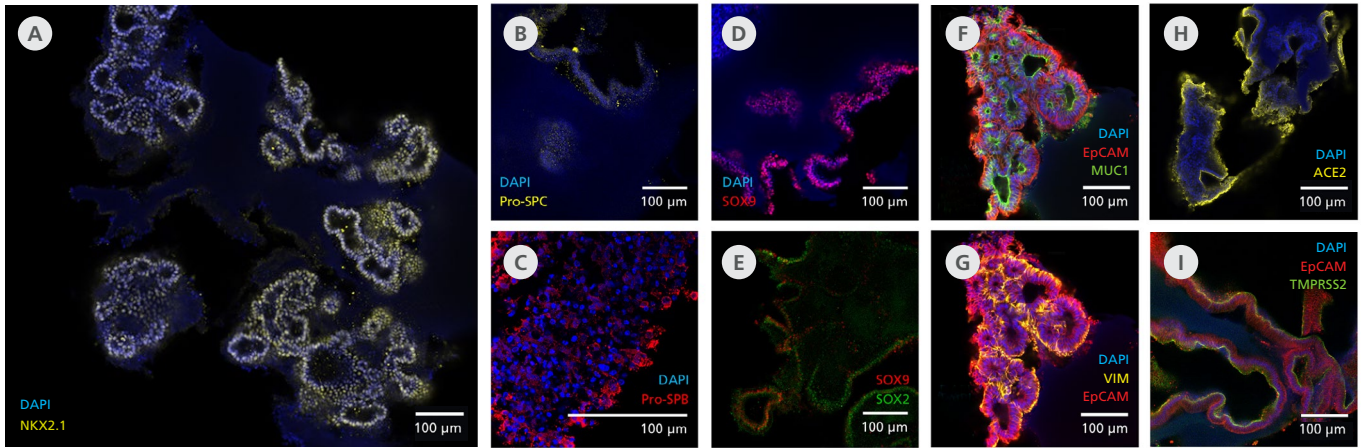


Figure 33. Branching Lung Organoids Cultured in STEMdiff™ Branching Lung Organoid Kit Feature Key Protein Markers and Exhibit Branching Morphogenesis

(A) Branching lung organoids express lung progenitor marker NKX2.1 throughout their branching structures and (B, C) demonstrate the presence of ATII-like cells with pro-surfactant protein B and C expressions. (D, E) These organoids undergo proximodistal differentiation demonstrated by the differential expression of SOX2 and SOX9. (F) MUC1 can be found luminally expressed while the (G) organoids are surrounded by VIM-expressing mesenchyme. (H, I) Branching lung organoids generated with the STEMdiff™ Branching Lung Organoid Kit also express proteins associated with SARS-CoV-2 entry, ACE2, and TMPRSS2. Protein expression was visualized by immunohistochemistry and confocal microscopy of branching lung organoids on Day 63.

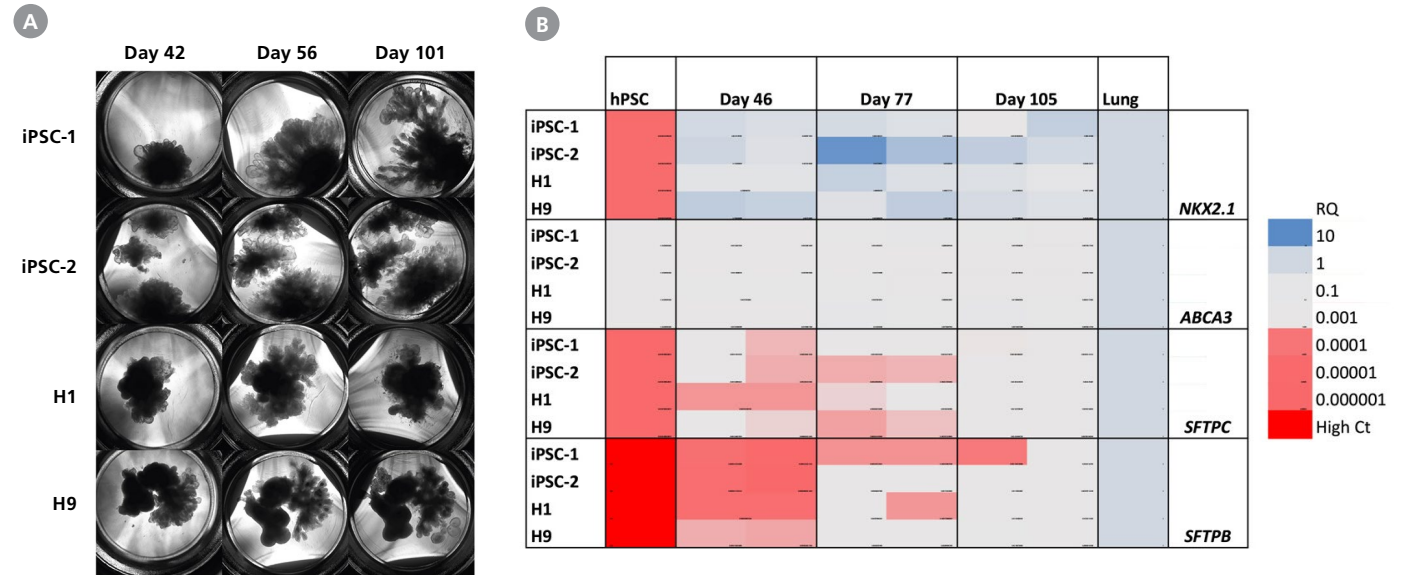


Figure 34. Branching Lung Organoids Cultured in STEMdiff™ Branching Lung Organoid Maturation Kit for Extended Periods Express More Mature Lung Markers

(A) The branching tips of branching lung organoids derived from peripheral blood endothelial progenitor cell-derived iPS cells (iPSC-1), peripheral blood mononuclear cell-derived iPS cells (iPSC-2), or embryonic stem cells (H1 and H9) continue to grow and branch up to day 101. (B) The expression levels of more mature distal lung markers SFTPC and SFTPB increase over time. Morphology and gene expression of branching lung organoids cultured up to day 105 were assessed by RT-qPCR. RQ-values were normalized to TBP and compared to commercially available lung RNA.

Airway Culture Training

Navigating new culture methods comes with steep learning curves. STEMCELL's airway culture experts can help you learn key techniques to increase your chances of success and get you off to a faster start. Enroll in one of our training programs below to build the foundational skills for airway culture.



Human Airway Epithelial Cell Training Course

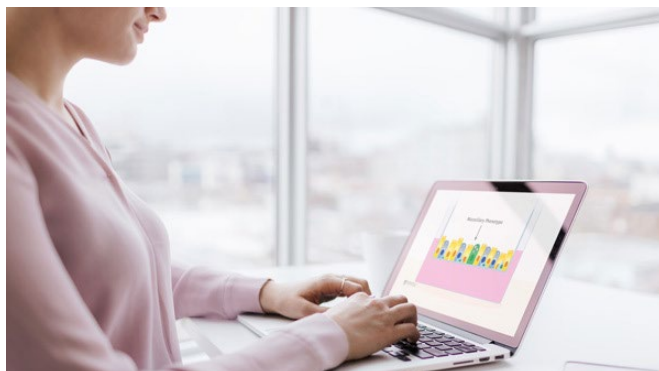
In this two-day, hands-on training course, STEMCELL's in-house airway culture experts guide participants on how to culture human airway epithelial cells (HAECs) at the ALI. Participants will learn how to expand primary HAECs, prepare cultureware for ALI culture, establish cultures of HAECs, and perform downstream assays at the ALI.



COURSE

Register for the Human Airway Epithelial Cell Training Course

www.stemcell.com/HAEC-Training-Course



On-Demand Pulmonary Course

Receive free virtual training at your own pace. Through a series of lectures, technical lab videos, and assessments, participants will learn the key concepts of how to model the human airway by expansion and differentiation of human airway epithelial cells at the ALI.



COURSE

Begin On-Demand Pulmonary Training

www.stemcell.com/On-Demand-Pulmonary

Product Information

Products for Expansion of Human Airway Epithelial Cells

Product	Catalog #
PneumaCult™-Ex Plus Medium	05040
PneumaCult™-Ex Medium	05008

Products for Differentiation of Human Airway Epithelial Cells at the ALI

Product	Catalog #
PneumaCult™-ALI Medium	05001
PneumaCult™-ALI-S Medium	05050

Products to Support Airway Cultures

Product	Catalog #
Costar® 12 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts	38023
Costar® 6.5 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts	38024
HTS Transwell®-96, 0.4 µm Pore Polyester Membrane Inserts	100-0419

For your convenience, PneumaCult™-ALI Medium can be purchased with Transwell® Inserts either as a 12-well format with 12 mm Transwell® Inserts, or as a 24-well format with 6.5 mm Transwell® Inserts.

Product	Catalog #
PneumaCult™-ALI Medium with 12 mm Transwell® Inserts	05021
PneumaCult™-ALI Medium with 6.5 mm Transwell® Inserts	05022

Products for Human Airway & Alveolar Organoids

Product	Catalog #
PneumaCult™ Airway Organoid Kit	05060
PneumaCult™ Apical-Out Airway Organoid Medium	100-0620
PneumaCult™ Alveolar Organoid Expansion Media	100-0847
PneumaCult™ Alveolar Organoid Differentiation Media	100-0861

To generate lung progenitor cells or branching lung organoids from ES and iPS cells, explore our STEMdiff™ products for respiratory research.

Related Products for hPSC-Derived Lung Models

Product	Catalog #
STEMdiff™ Lung Progenitor Kit	100-0230
STEMdiff™ Branching Lung Organoid Kit	100-0195
mTeSR™1	85850
mTeSR™ Plus	05825



RESOURCE

Learn More About Airway Modeling
www.stemcell.com/Airway-Modeling



WALLCHART

Cellular Organization and Biology of the Respiratory System
www.stemcell.com/RespiratoryWallchart

Supplementary Reagents

Cell Culture Supplements

STEMCELL's cell culture supplements can be used in various stages of in vitro airway culture, including dissociation, expansion, and differentiation.

Product	Size	Catalog #
Animal Component-Free Cell Dissociation Kit	1 Kit	05426
Heparin Solution	2 mL	07980
Hydrocortisone Stock Solution	3 mL	07925
	10 x 3 mL	07926

Cryopreservation Media

Cryopreservation is an important part of the pulmonary research workflow. CryoStor® CS10 is a serum- and animal component-free freezing medium, and provides a safe and protective environment for cells during the freezing, storage, and thawing processes.

Antibodies

Analyze cells with antibodies that are verified to work with STEMCELL's airway epithelial cell culture reagents for select applications

Cytokines

Activate, expand, or differentiate your cells with cytokines, chemokines, and growth factors across a variety of applications, including airway epithelial cell research.

Cell Dyes and Stains

GloCell™ Dyes for Live/Dead Cell Staining

GloCell™ Fixable Viability Dyes are fluorescent amine-labeling dyes for live/dead staining of mammalian cells, allowing clear exclusion of dead cells from flow cytometry data. These dyes are resistant to washing and fixation and are compatible with intracellular antibody staining protocols.

Annexin V Dyes

Annexin V is a characteristic cell death marker that can be used to detect early and late-stage apoptotic mammalian cells.



PRODUCT

Learn More About GloCell™ Dyes
www.stemcell.com/GloCell



PRODUCT

Learn More About Annexin V Dyes
www.stemcell.com/AnnexinV



PRODUCT

View a Complete Listing of Available Antibodies
www.stemcell.com/Antibodies



PRODUCT

View a Complete Listing of Available Cytokines
www.stemcell.com/Cytokines

Tools for COVID-19 Research

Recombinant Proteins

Product	Size	Catalog #
SARS-CoV-2 Recombinant Nucleocapsid Protein, aa1-419 (E. coli-expressed)	100 µg	100-0590
	1000 µg	100-0591
SARS-CoV-2 Recombinant Nucleocapsid Protein, aa1-419 (HEK293-expressed)	100 µg	100-0592
	1000 µg	100-0593
SARS-CoV-2 Recombinant Spike Protein, aa16-685 (HEK293-expressed)	100 µg	100-0594
	1000 µg	100-0594
SARS-CoV-2 Recombinant Spike Protein, aa319-541 (Yeast-expressed)	100 µg	100-0596
	1000 µg	100-0597
Human Recombinant ACE2 Protein, aa18-740 (HEK293-expressed)	100 µg	100-0598
	500 µg	100-0599

Primary Antibodies

Product	Size	Catalog #
Anti-SARS-CoV Nucleoprotein Antibody, Clone 001 (Recombinant)	50 µL	100-0529
	100 µL	100-0580
Anti-SARS-CoV Spike Protein S1 Receptor-Binding Domain Antibody, Clone D005 (Recombinant)	50 µL	100-0581
	100 µL	100-0582
Anti-SARS-CoV-2 Spike Protein S1 Receptor-Binding Domain Antibody, Clone Covi-1 (Blocking/Recombinant)	100 µL	100-0583
Anti-SARS-CoV-2 Spike Protein S1 Receptor-Binding Domain Antibody, Clone Covi-2 (Blocking/Recombinant)	100 µL	100-0584

ELISA Kits

Product	Catalog #
Human SARS-CoV-2 Nucleoprotein IgG Antibody ELISA Kit	100-0686
Human ACE2 ELISA Kit	100-0687
Mouse ACE2 ELISA Kit	100-0688
Human CD13 (ANPEP) ELISA Kit	100-0689

Peptide Substrates for Detection of Coronavirus Proteases

Product	Size	Catalog #
CoV Protease Substrate-1 TF5	100 tests	100-0505
	1000 tests	100-0506
CoV Protease Substrate-1 EDANS	100 tests	100-0507
	1000 tests	100-0508
CoV Protease Substrate-2 EDANS	100 tests	100-0509
	1000 tests	100-0510
CoV Protease Substrate-2 IF670	100 tests	100-0511
	1000 tests	100-0512

Screening Kits

Product	Catalog #
Human SARS-CoV-2 Spike Protein Inhibitor Screening Kit	100-0700

Viral Peptide Pools

Product	Size	Catalog #
SARS-CoV-2 (Nucleocapsid Protein) Peptide Pool	25 µg/peptide	100-0647
SARS-CoV-2 (Spike Protein) Peptide Pool	25 µg/peptide	100-0676
SARS-CoV-2 (VME1) Peptide Pool	25 µg/peptide	100-0648
Influenza (HLA Class I Control) Peptide Pool	25 µg/peptide	100-0672
RSV (HLA Class I Control) Peptide Pool	25 µg/peptide	100-0674
EBV (EBNA-1) Peptide Pool	25 µg/peptide	100-0669
EBV (BZLF1) Peptide Pool	25 µg/peptide	100-0670
EBV (LMP2) Peptide Pool	25 µg/peptide	100-0671

For more information or to view our complete listing of viral peptide pools, please visit:

www.stemcell.com/viral-peptide-pools

References

1. Bérubé K et al. (2010) Human primary bronchial lung cell constructs: the new respiratory models. *Toxicology* 278(3): 311–8.
2. Prytherch Z et al. (2011) Tissue-specific stem cell differentiation in an in vitro airway model. *Macromol Biosci* 11(11): 1467–77.
3. Ostrowski LE et al. (2012) Interferon γ stimulates accumulation of gas phase nitric oxide in differentiated cultures of normal and cystic fibrosis airway epithelial cells. *Lung* 190(5): 563–71.
4. Comer D et al. (2013) Airway epithelial cell apoptosis and inflammation in COPD, smokers and nonsmokers. *Eur Respir J* 41(5): 1058–67.
5. Yan et al. (2017) Human bocavirus type-1 capsid facilitates the transduction of Ferret Airways by Adeno-Associated Virus Genomes. *Hum Gene Ther* 28(8): 612–25.
6. Tata et al. (2013) Dedifferentiation of committed epithelial cells into stem cells in vivo. *Nature* 503(7475): 218–23.
7. Dale et al. (2019) Rock inhibitor Y-27632 enables feeder-free, unlimited expansion of Sus Scrofa domesticus swine airway stem cells to facilitate respiratory research. *Stem Cells Int* 2019: 3010656.
8. Wethington D et al. (2019) Mathematical modeling identifies the role of adaptive immunity as a key controller of respiratory syncytial virus in cotton rats. *J R Soc Interface* 16(160): 20190389.
9. Engler et al. (2019) Isolation, maintenance and differentiation of primary tracheal basal cells from adult rhesus macaque. *Methods Protoc* 2(4): E79.
10. Matrosovich MN et al. (2004) Human and avian influenza viruses target different cell types in cultures of human airway epithelium. *Proc Natl Acad Sci U S A* 101(13): 4620–4.
11. Hao W et al. (2012) Infection and propagation of human rhinovirus c in human airway epithelial cells. *J Virol* 86(24): 13524–32.
12. Hiemstra PS et al. (2015) The innate immune function of airway epithelial cells in inflammatory lung disease. *Eur Respir J* 45(4): 1150–62.
13. Lin H et al. (2007) Air-liquid interface (ALI) culture of human bronchial epithelial cell monolayers as an in vitro model for airway drug transport studies. *J Pharm Sci* 96(2): 341–50.
14. Tralau T et al. (2012) Drug-mediated toxicity: Illuminating the ‘bad’ in the test tube by means of cellular assays? *Trends Pharmacol Sci* 33(7): 353–64.
15. Andreoli C et al. (2003) A review of in vitro methods to assess the biological activity of tobacco smoke with the aim of reducing the toxicity of smoke. *Toxicol in Vitro* 17(5-6): 587–94.
16. Balharry D et al. (2008) An in vitro approach to assess the toxicity of inhaled tobacco smoke components: nicotine, cadmium, formaldehyde and urethane. *Toxicology* 244(1): 66–76.
17. Crystal R et al. (2008) Airway epithelial cells: current concepts and challenges. *Proc Am Thorac Soc* 5(7): 772–7.
18. Guoqing W et al. (2019) Characterization of an immortalized human small airway basal stem/progenitor cell line with airway region-specific differentiation capacity. *Respir Res* 20: 196.
19. Yang J et al. (2017) Smoking-dependent distal-to-proximal repatterning of the adult human small airway epithelium. *Am J Respir Cell Mol Biol* 196(3): 340–52.

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and Alveolar Epithelial Cells



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DOCUMENT #27188 VERSION 2.0.2 FEB 2024

