AIRWAY RESEARCH

PneumaCult™ Culture Media for Human Airway Epithelial Cells

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In Vitro Human Airway Model Systems

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. 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 epithelial 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 organoids represent another versatile system for human airway modeling. These 'mini-airways' accurately replicate the histological and functional aspects of the in vivo tissue and their cellular morphology is physiologically similar to the human airway epithelium. By developing these structures and functions, the airway organoid emulates the in vivo physiological or pathological environment, making it useful for modeling respiratory diseases. Compared to ALI cultures, human airway organoids are generated using a cell culture insert-free protocol, facilitating high-throughput drug screening.

PneumaCult™ Culture System

The PneumaCult™ culture system consists of serum- and bovine pituitary extract (BPE)-free culture media for in vitro human airway modeling. While primary human bronchial epithelial cells (HBECs) or human small airway epithelial cells (HSAECs) can be expanded in submerged culture with PneumaCult™-Ex Plus Medium, ALI cultures of differentiated HBECs or HSAECs can be achieved with PneumaCult™-ALI Medium or PneumaCult™-ALI-S Medium, respectively. For differentiation as 3D airway organoids, PneumaCult™ Airway Organoid Kit is recommended. 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 In Vitro Airway Model Systems?

Airway organoids and organotypic models like ALI culture hold enormous potential for respiratory research, with a wide range of experimental uses. This includes a number of highly specialized applications for which submerged culture techniques provide inadequate models. Use airway organoids or ALI cultures of airway epithelial cells to:

STUDY THE CELL BIOLOGY OF THE RESPIRATORY EPITHELIUM. ALI culture is the most physiologically relevant model for studying the respiratory epithelium in vitro.[2] Airway organoids, with a centralized lumen surrounded by a polarized airway epithelial cell layer, also provide a functional in vitro culture system for studying the airway epithelium.

MODEL RESPIRATORY DISEASES. Airway epithelial cells from patients with chronic respiratory diseases such as cystic fibrosis, COPD, asthma, idiopathic pulmonary fibrosis, lung cancer, and infectious diseases can be cultured, enabling disease mechanisms to be studied in vitro.[3,4] Researchers considering 3D models can also use patient-derived airway organoids to study CF. These organoids demonstrate suitability for monitoring cystic fibrosis transmembrane conductance regulator (CFTR) function using the forskolin-induced swelling assay.

STUDY VIRAL OR BACTERIAL INFECTION OF THE RESPIRATORY EPITHELIUM. Some respiratory viruses selectively target cell types present only in fully differentiated airway cell cultures.[10,11] In vitro airway models can also be used to study the host defense provided by airway epithelium against bacterial and viral infections.[12]

Find out more about why ALI is a physiologically relevant model system for studying viral infection, including COVID-19 at **www.stemcell.com/studying-covid-19-with-ali-cultures**.

TEST DRUG FORMULATIONS FOR INHALATION DELIVERY. Aerosol particles can be directly deposited onto the semi-dry apical cell surface, mimicking the deposition of powders onto the lung surface in vivo, for ALI cultures.[13,14]

TEST TOXICITY OF INHALED SUBSTANCES. Responses of ALI-differentiated primary epithelial cells to insults such as tobacco smoke components closely mimic reported changes in the human airway.[15,16]

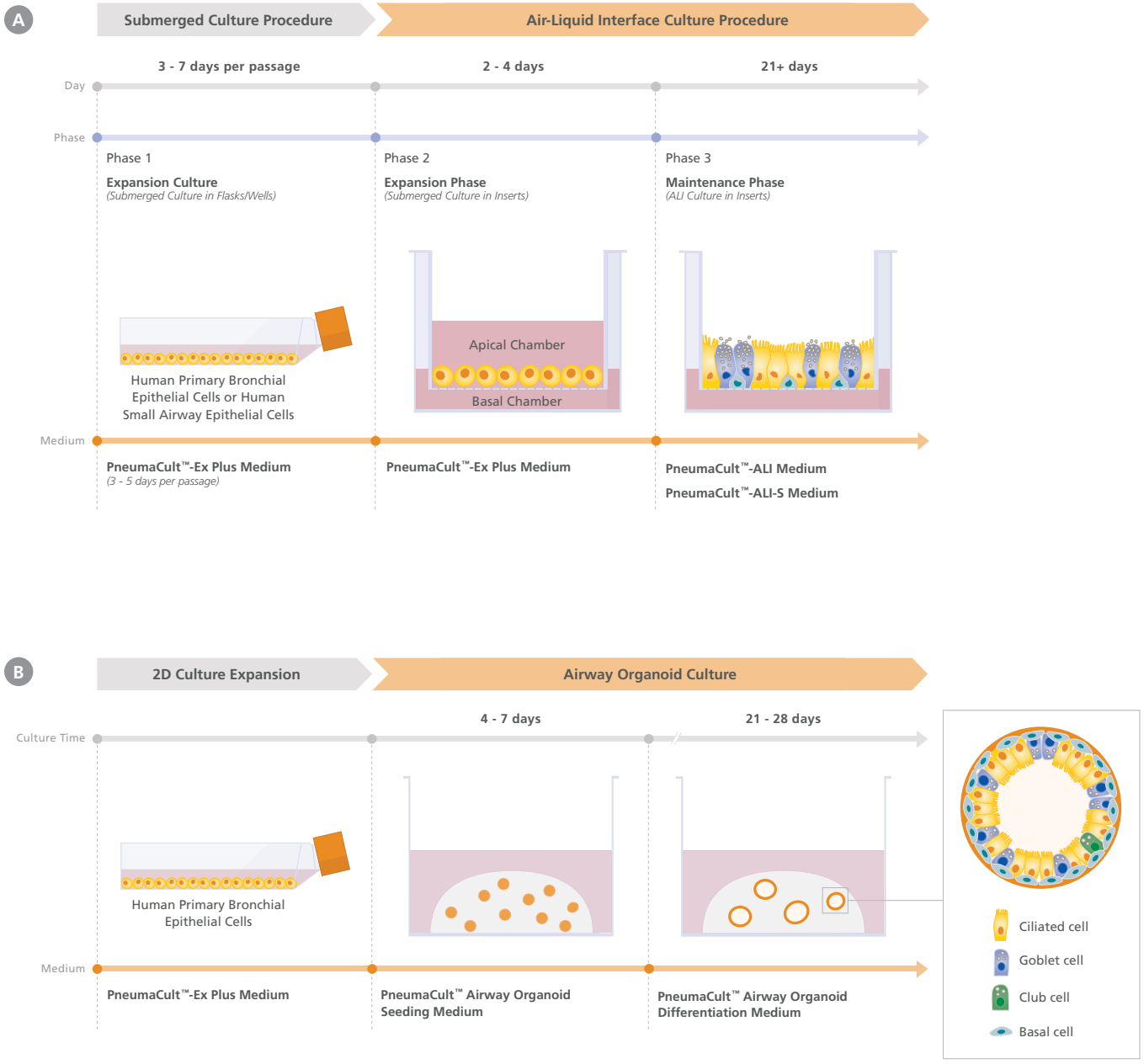


Figure 1. Overview of the PneumaCult™ Culture System

(A) 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 obtained following 21 - 28 days of incubation and can be maintained for more than one year. (B) 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.

STEMCELL Products for In Vitro Airway Cultures

Dissociation	Expansion	Differentiation	Cryopreservation
• Animal Component-Free Cell Dissociation Kit • Gentle Cell Dissociation Reagent	• PneumaCult™-Ex Plus Medium • PneumaCult™-Ex Medium • Hydrocortisone Stock Solution	• PneumaCult™-ALI Medium: Large airway cultures • PneumaCult™-ALI-S Medium: Small airway cultures • PneumaCult™ Airway Organoid Kit • Hydrocortisone Stock Solution • Heparin Solution • HTS Transwell®-96, 0.4 µm Pore Polyester Membrane Inserts • Costar® 6.5 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts • Costar® 12 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts	• CryoStor® CS10 (For expansion phase only)

Related Products for Respiratory Research

In vitro lung models can also be generated from human pluripotent stem cells (hPSCs) following a stepwise directed differentiation. Researchers without access to primary human airway epithelial cells (HAECs) could use these models for studying lung development and respiratory diseases. Current protocols for hPSC-derived lung models are highly variable and lack standardization between different labs. STEMCELL Technologies has developed cell culture media and streamlined protocols for the reproducible generation of lung progenitors and lung organoids across multiple human embryonic stem (ES) and induced pluripotent stem (iPS) cell lines. Learn more at **www.stemdiff.com**.

PRODUCT

Learn about STEMdiff™ Lung Progenitor Kit

www.stemcell.com/stemdiff-lung-progenitor

PRODUCT

Explore the STEMdiff™ Branching Lung Organoid Kit

www.stemcell.com/stemdiff-branching-lung-organoid

Expansion of Human Airway Epithelial Cells

PneumaCult™-Ex Plus Medium

Current feeder-free expansion media for culturing 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 air-liquid interface or airway organoid culture (Figure 1). 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?

Commercially available primary HBECs at passage 1 (P1) or HSAECs at passage 2 (P2) were thawed and seeded into T-25 cm^2 flasks containing PneumaCult™-Ex Plus Medium, 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.

HBECs cultured in PneumaCult™-Ex Plus Medium experience at least two more population doublings compared to those cultured in Bronchial Epithelial Growth Medium (Figure 2). Similarly, HSAECs cultured in PneumaCult™-Ex Plus Medium exhibit a higher rate of proliferation (Figure 3) 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 (Figure 4) that express higher levels of basal cell markers CD49f and CD271 (Figures 5 and 6). The maintenance of stem-like basal cells in PneumaCult™-Ex Plus Medium permits better ALI differentiation potential even after extended passaging.

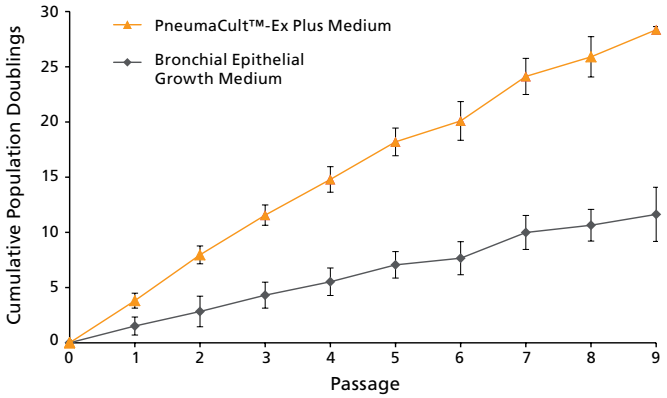


Figure 2. Human Bronchial Epithelial Cells Cultured in PneumaCult™-Ex Plus Medium Expand Faster

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

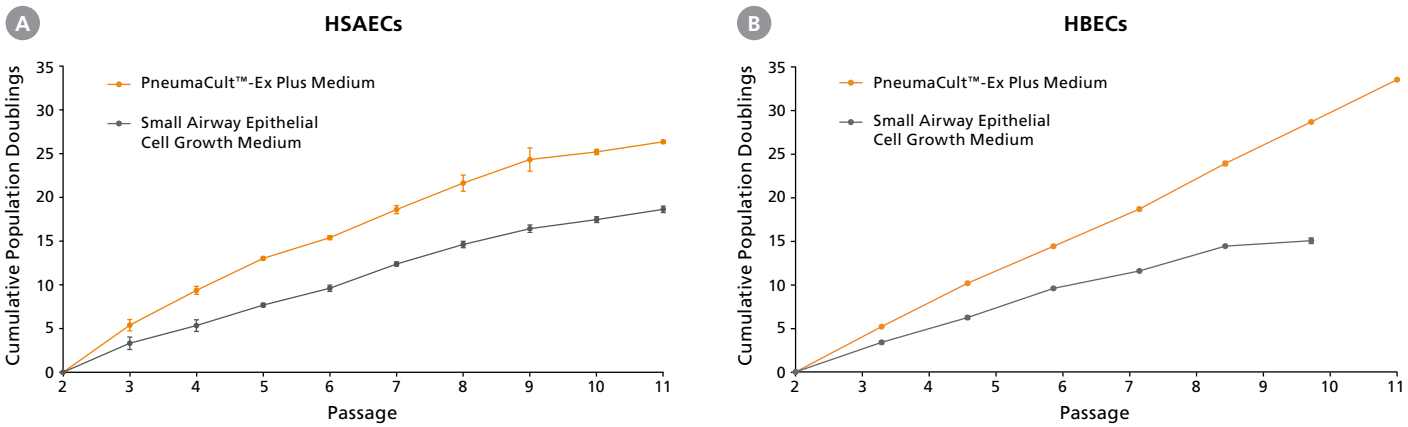


Figure 3. Human Small Airway Epithelial Cells and Human Bronchial Epithelial Cells Grow at a Higher Rate During Expansion When Cultured in PneumaCult™-Ex Plus Medium

(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.

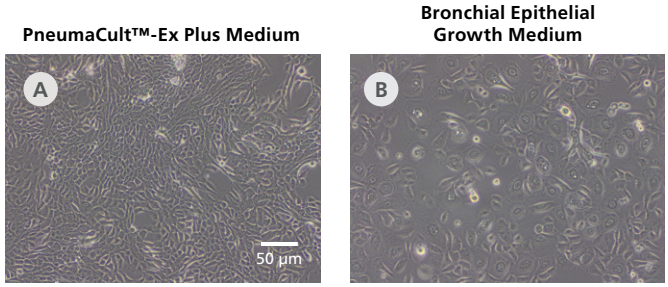


Figure 4. Human Bronchial Epithelial Cells Cultured in PneumaCult™-Ex Plus Medium Are Tightly Packed

Representative live culture images for passage 4 (P4) HBECs cultured in (A) PneumaCult™-Ex Plus Medium or (B) Bronchial Epithelial Growth Medium. All images were taken using a 10X objective. Scale bar = 50 µm.

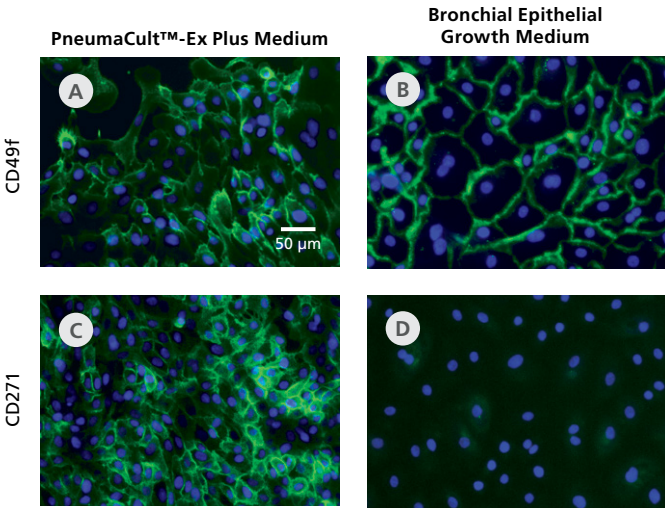


Figure 5. Human Bronchial Epithelial Cells in PneumaCult™-Ex Plus Medium Maintain Basal Cell Marker Expression

Basal cell markers (A,B) CD49f and (C,D) CD271 for passage 4 HBECs cultured in (A,C) PneumaCult™-Ex Plus Medium and (B,D) Bronchial Epithelial Growth Medium were detected by immunocytochemistry. The basal cells are marked with antibody stains (green). The nuclei are counterstained with DAPI (blue). All images were taken using a 10X objective. Scale bar = 50 µm.

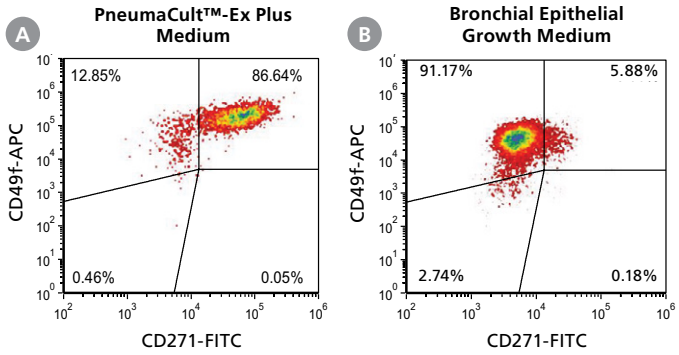


Figure 6. Culturing Human Bronchial Epithelial Cells in PneumaCult™-Ex Plus Medium Results in a Higher Proportion of Basal Cells

Passage 4 HBECs cultured in (A) PneumaCult™-Ex Plus Medium and (B) Bronchial Epithelial Growth Medium were characterized by flow cytometry to detect expression of the basal cell markers CD49f and CD271. HBECs cultured in PneumaCult™-Ex Plus Medium have a higher proportion of cells co-expressing CD49f and CD271, compared to those cultured in Bronchial Epithelial Growth Medium.

Differentiation potential of HBECs expanded in PneumaCult™-Ex Plus Medium or Bronchial Epithelial Growth Medium was assessed by seeding them in ALI culture using PneumaCult™-ALI Medium.

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 7).

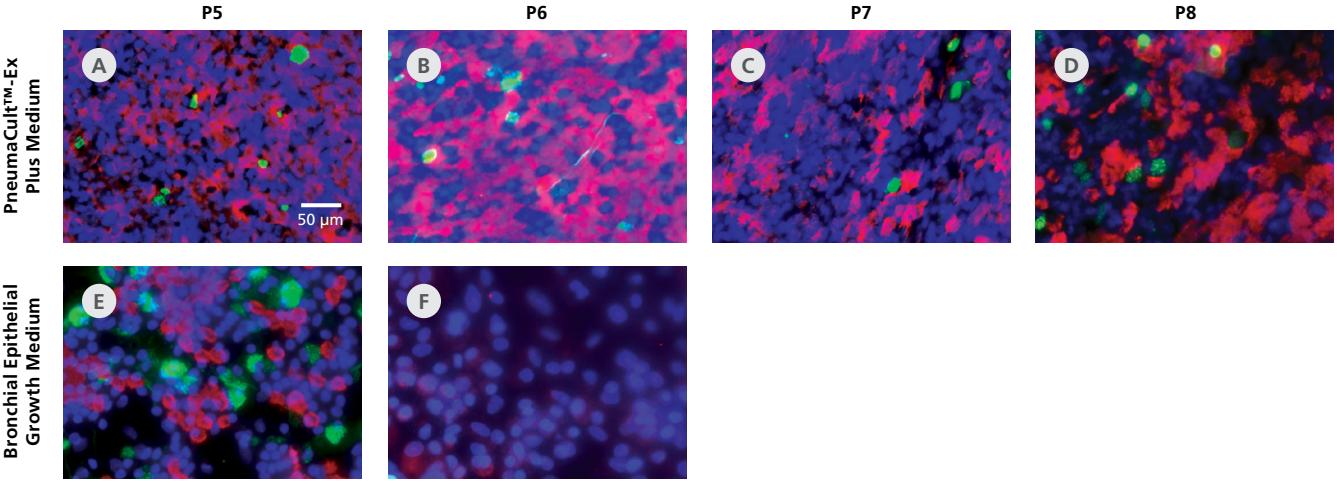


Figure 7. Human Bronchial Epithelial Cells Cultured in PneumaCult™-Ex Plus Medium Followed by PneumaCult™-ALI Medium Demonstrate Improved Pseudostratified Mucociliary Differentiation

P4 HBECs were seeded and passaged using (A-D) PneumaCult™-Ex Plus Medium or (E-F) Bronchial Epithelial Growth Medium, followed by ALI differentiation at passages 5 to 8 with the use of PneumaCult™-ALI Medium. 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. Scale bar = 50 µm.

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, indicated by higher TEER values at each passage (Figure 8A). 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 8B).

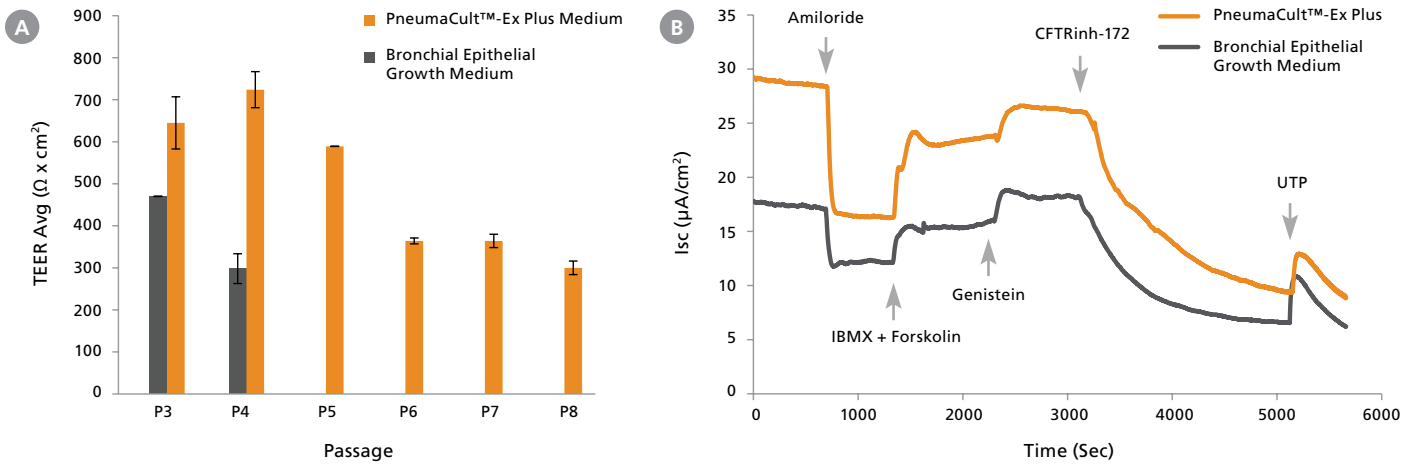


Figure 8. Human Bronchial Epithelial Cells Expanded in PneumaCult™-Ex Plus Medium Showed Better Barrier Integrity and Function in Comparison with Bronchial Epithelial Growth Medium

(A) TEER measurements 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 Medium or Bronchial Epithelial Growth Medium. Amiloride: ENaC inhibitor; IBMX and Forskolin: CFTR activators; Genistein: CFTR potentiator; CFTRinh-172: CFTR inhibitor; UTP: calcium-activated chloride channels (CaCCs) activator. All ALI differentiation cultures were performed using PneumaCult™-ALI Medium.

PROTOCOL
Expansion of HBECs
www.stemcell.com/HBEC-expansion

PROTOCOL
Measure TEER in ALI cultures
www.stemcell.com/TEER-protocol

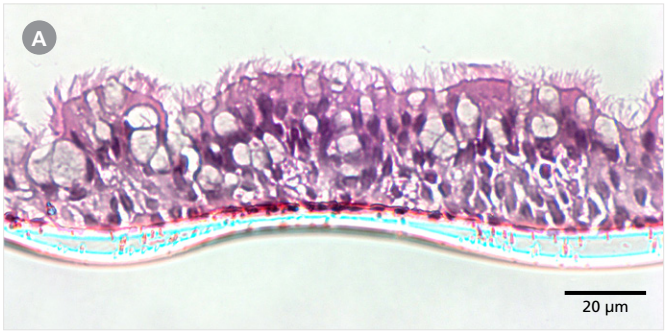
Differentiation of Human Airway Epithelial Cells at the Air-Liquid Interface

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, including ciliated cells, mucus-secreting goblet cells, and apical tight junctions (Figure 10).

An important function of the large airway (tracheobronchial epithelium) 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 8A).



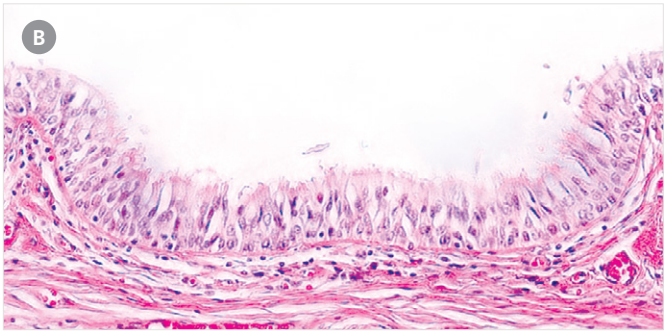


Figure 9. Primary Human Bronchial Epithelial Cells Cultured at the Air-Liquid Interface Recapitulate the In Vivo Bronchial Epithelium

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.

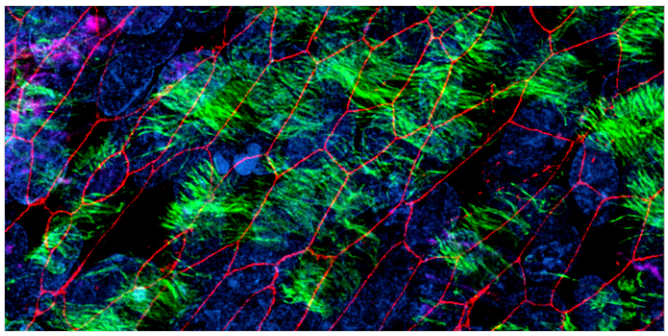


Figure 10. PneumaCult™-ALI Medium Enables Differentiation of Human Bronchial Epithelial Cells to Cell Types Present in the Large Airway Epithelium

Confocal image of whole mount immunostained ALI culture showing P2 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). All images were 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).

Why Use PneumaCult™-ALI-S Medium?

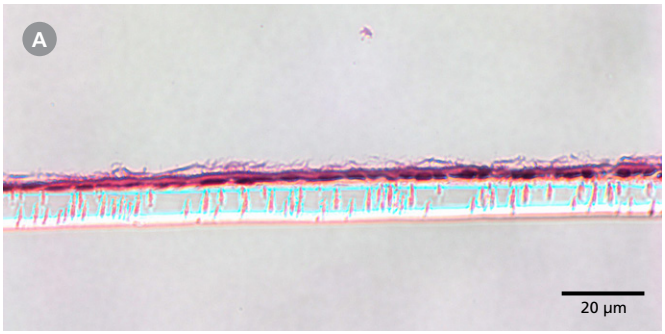
REGIONAL SPECIFICITY. Differentiate to ALI cultures with morphology and cell type ratio representative of the small airway epithelium.

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

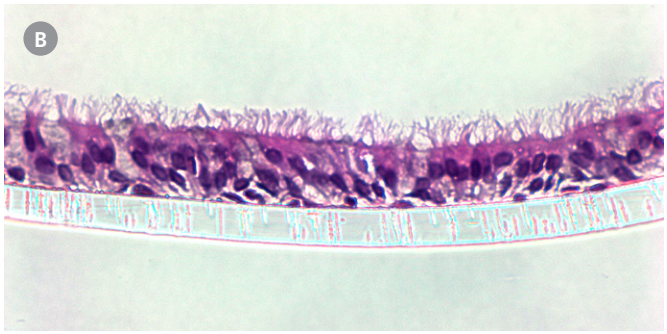


Figure 11. Human Small Airway Epithelial Cells Cultured at the Air-Liquid Interface Using PneumaCult™-ALI-S Medium Recapitulate the Small Airway Epithelium

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 (P3), 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.

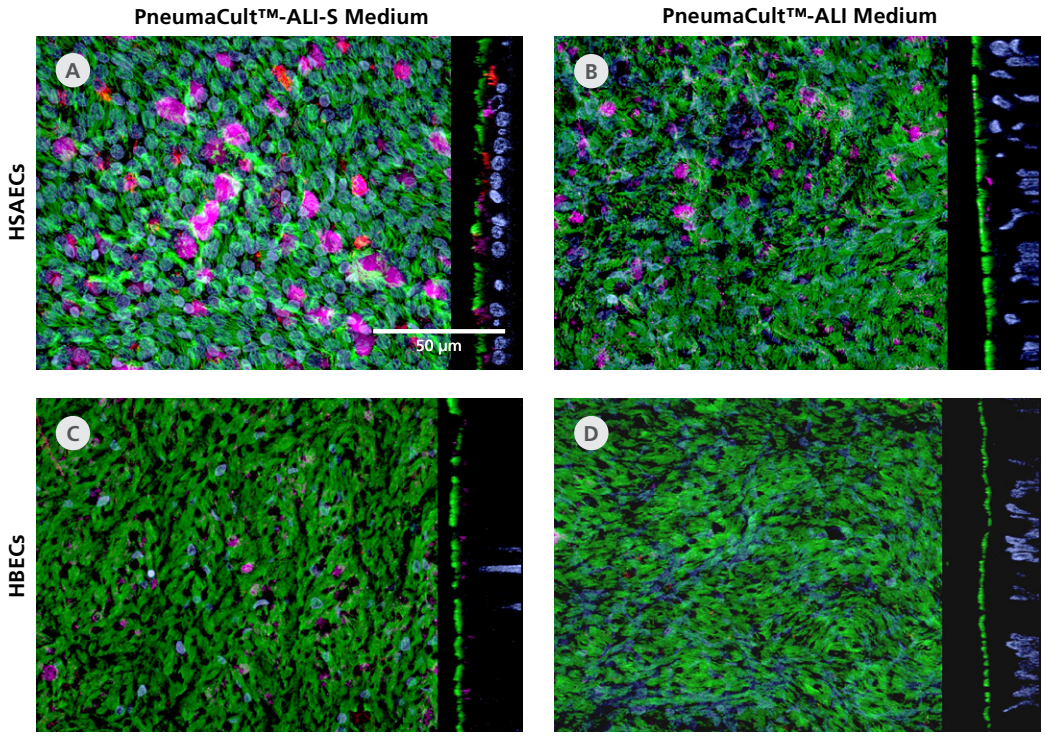


Figure 12. PneumaCult™-ALI-S Medium Enables Differentiation of Human Small Airway Epithelial Cells to Cell Types Present in the Small Airway Epithelium

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 P3, 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.

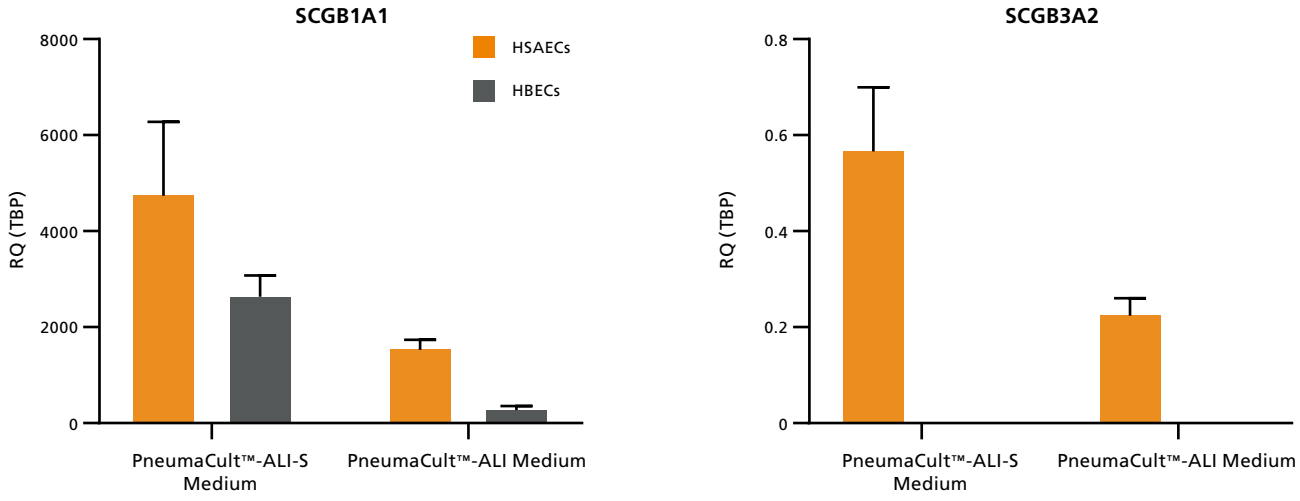


Figure 13. Human Small Airway Epithelial Cells Cultured in PneumaCult™-ALI-S Medium Express High Levels of Small Airway Epithelium Markers

HSAECs and HBECs expanded in PneumaCult™-Ex Plus Medium and differentiated in PneumaCult™-ALI-S Medium or PneumaCult™-ALI Medium at P3. After 28 days of differentiation, the ALI cultures were analysed 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 14), as well as TEER values that are indicative of optimal culture differentiation maturity (Figure 15).

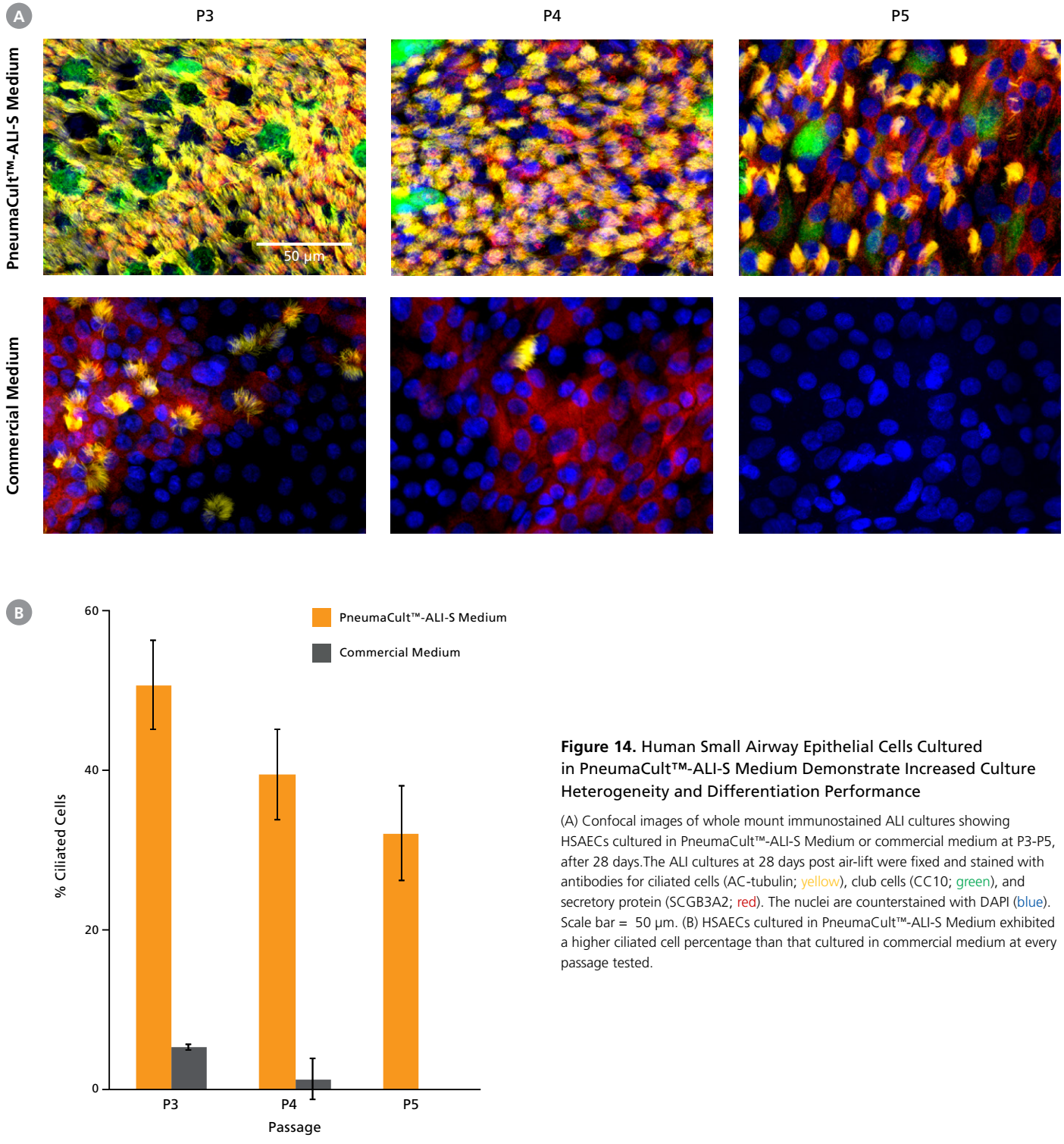


Figure 14. Human Small Airway Epithelial Cells Cultured in PneumaCult™-ALI-S Medium Demonstrate Increased Culture Heterogeneity and Differentiation Performance

(A) Confocal images of whole mount immunostained ALI cultures showing HSAECs cultured in PneumaCult™-ALI-S Medium or commercial medium at P3-P5, after 28 days.The ALI cultures at 28 days post air-lift were fixed and stained with antibodies for ciliated cells (AC-tubulin; yellow), club cells (CC10; green), and secretory protein (SCGB3A2; red). The nuclei are counterstained with DAPI (blue). Scale bar = 50 µm. (B) HSAECs cultured in PneumaCult™-ALI-S Medium exhibited a higher ciliated cell percentage than that cultured in commercial medium at every passage tested.

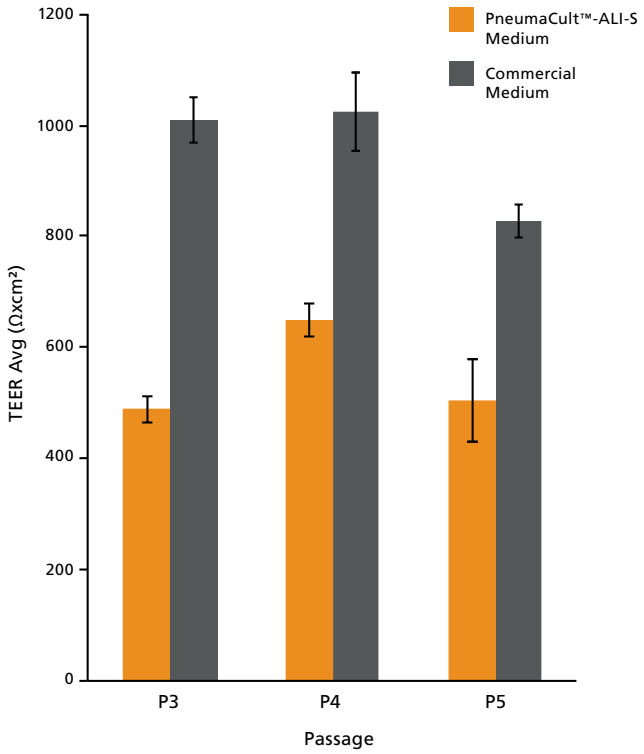


Figure 15. Human Small Airway Epithelial Cells Cultured in PneumaCult™-ALI-S Medium Display Optimal Culture Differentiation and Maturity

TEER measurements for ALI cultures at 28 days post air-lift using HSAECs expanded in PneumaCult™-Ex Plus Medium and differentiated using PneumaCult™-ALI-S Medium or commercial medium. HSAECs cultured in PneumaCult™-ALI-S Medium display TEER values that are indicative of optimal culture differentiation maturity compared to those cultured in commercial medium

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

Transwell® Inserts

Transwell® Inserts are recommended for culturing airway epithelial cells at the ALI and have been validated for use with PneumaCult™-ALI Medium.

The performance of ALI cultures can be greatly affected by the quality and type of culture inserts used to set up the assay. In a side-by-side comparison between Transwell® inserts and alternative commercially available inserts of the same material and pore size, ALI cultures generated using Transwell® Inserts were more differentiated when primary HBECs were cultured with PneumaCult™-ALI Medium (Figure 16). qPCR analysis also demonstrated a higher expression of goblet and ciliated cell markers in the ALI cultures generated with Transwell® Inserts (Figure 17).

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

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

REPRODUCIBLE. Maximize experimental reproducibility with low lot-to-lot variability, complementary to the PneumaCult™ formulation.

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

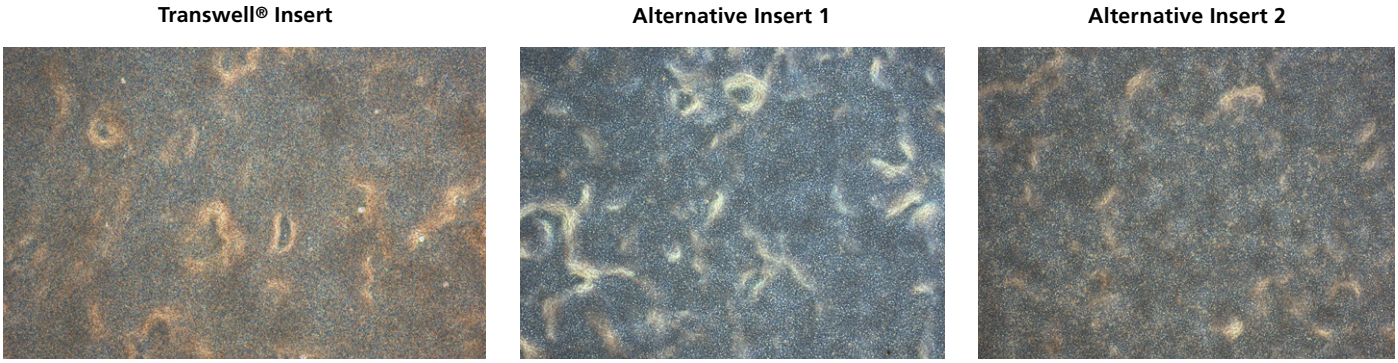


Figure 16. Air-Liquid Cultures of Human Bronchial Epithelial Cells Generated Using Transwell® Inserts are More Differentiated

HBECs expanded in PneumaCult™-Ex Medium were seeded onto the inserts at P3 and differentiated in PneumaCult™-ALI Medium for 21 days. Comparison was made with alternative commercially available inserts of the same material and pore size.

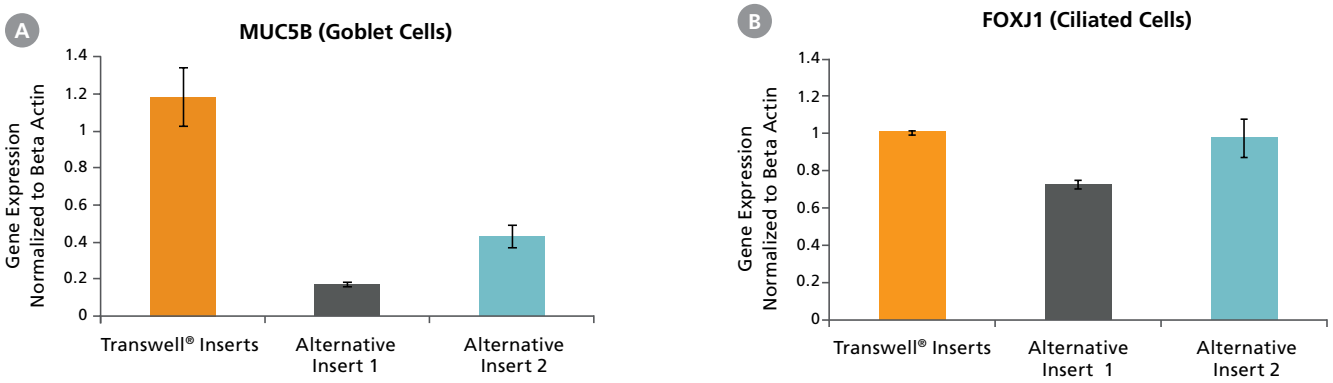


Figure 17. Air-Liquid Cultures of Human Bronchial Epithelial Cells Generated Using Transwell® Inserts Show Higher Differential Epithelial Cell Marker Expression

HBECs expanded in PneumaCult™-Ex Medium were seeded onto the inserts at P3 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.

Differentiation of Human Airway Epithelial Cells as Airway Organoids

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 18).

RESOURCE

Learn about lung organoid cultures
www.stemcell.com/lung-organoids

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 HAECs at P1 from both healthy and CF donors were expanded and serially passaged using PneumaCult™-Ex Plus Medium. At passages 2 to 5, 2 x 10^3 HAECs were embedded into a 50 µL Matrigel® dome using PneumaCult™ Airway Organoid Seeding Medium (Figure 18A). After 7 days of expansion, the medium was switched to PneumaCult™ Airway Organoid Differentiation Medium (Figure 18B). Following differentiation, the organoids demonstrated lumens and robust cilia beating inside the lumen (Figure 18C).

Expansion in PneumaCult™ Airway Organoid Seeding Medium

Differentiation in PneumaCult™ Airway Organoid Differentiation Medium

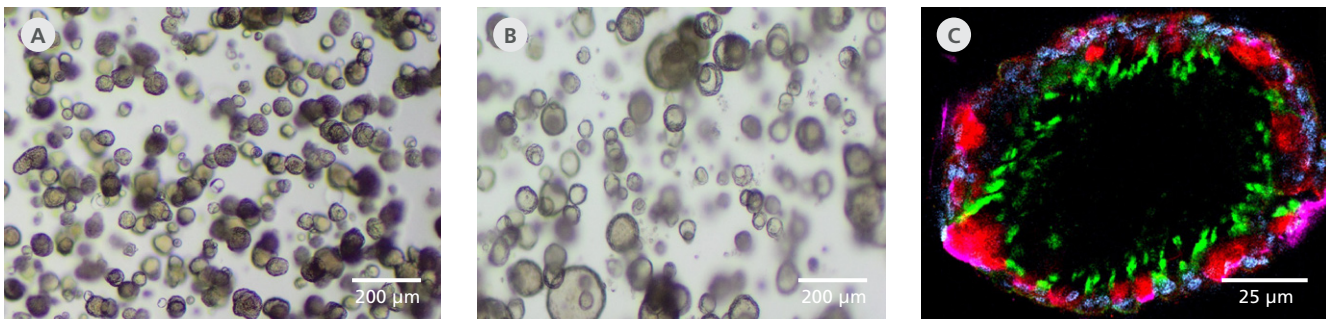


Figure 18. Fully Differentiated Human Airway Organoids Can Be Generated Using PneumaCult™ Airway Organoid Kit

(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 µm and (B) 25 µm.

Fully differentiated airway organoids, grown from both healthy and CF donors using the PneumaCult™ Airway Organoid Kit, were treated with either dimethyl sulfoxide (DMSO) control or 20 µM amiloride, 10 µM forskolin, and 25 µM genistein, and allowed to incubate for 6 hours to bring about forskolin-induced swelling. The surface area of the organoids was measured (Figure 19A and 19B). Organoids were also collected in cold Corning® Cell Recovery Solution. Following their enzymatic dissociation in ACCUTASE™ into a single-cell suspension, the cells were quantified for the number of ciliated cells. The ciliated cell percentage in organoids grown from both healthy (Figure 20A) and CF donors (Figure 20B) increased from P3 to P5.

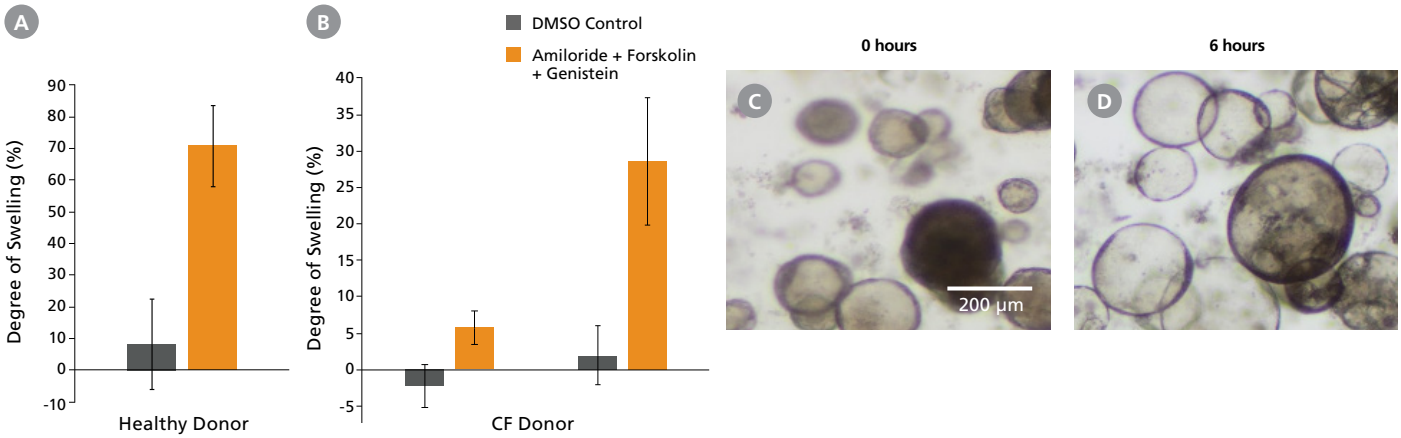


Figure 19. Airway Organoids are Suitable for Assessing CFTR Protein Expression Using Forskolin-Induced Swelling Assay

(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 ± 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 µm.

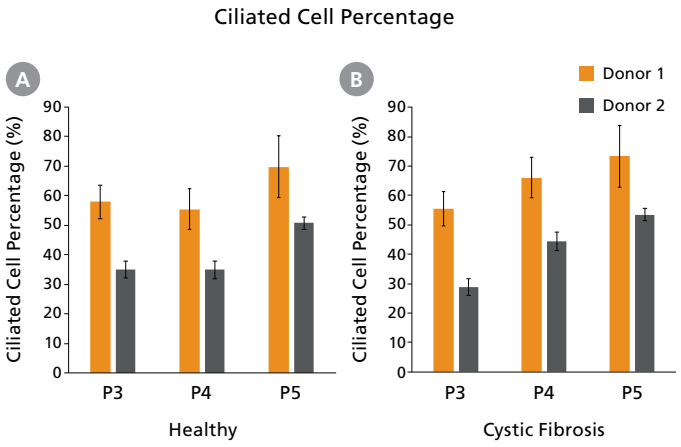


Figure 20. Fully Differentiated Airway Organoids Retain Morphological Characteristics at Different Passages

The ciliated cell percentage in organoids grown from (A) healthy and (B) CF donors using PneumaCult™ Airway Organoid Kit increased from P3 to P5. The total number of cells and the number of ciliated cells were counted using a hemocytometer. Error bars represent ± 95% confidence interval for the mean (n=3).

Contract Assay Services

Obtain timely and clinically relevant data with the help of our in-house experts at Contract Assay Service (CAS), a contract research organization within STEMCELL Technologies.

Pulmonary Services Using ALI Culture

To help you evaluate the effects of your investigative compounds on the human airway, CAS offers services using PneumaCult™-based ALI cultures of airway epithelial cells.

Use ALI cultures of airway epithelial cells in customized services from CAS to:

- Study respiratory epithelium cell biology
- Model respiratory diseases
- Study respiratory epithelium infection

CAS also offers ALI culture-based services to evaluate the effect of client-submitted test or reference compounds on the modulation of epithelial barrier function.

To learn more about CAS, visit **www.contractassay.com**.

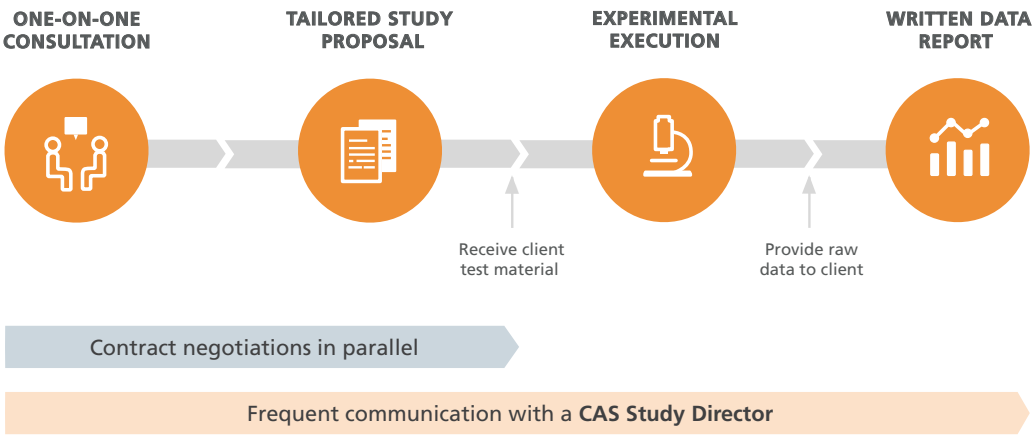


Figure 21. Overview of STEMCELL's Contract Assay Services Workflow

A designated CAS Study Director will initiate discussions about your data requirements. Based on your feedback and in consultation with in-house experts, a tailored study proposal with clearly defined project objectives, scope, timeline, and cost will be designed. After execution of the appropriate contracts and confidentiality agreements, the project will move to the implementation phase where a dedicated team of scientists will initiate experiments and data analysis. Throughout this process, you will be in clear and frequent communication with the CAS Study Director and other experts from the team. Study data and reports will be shared with you in a timely manner via a secure cloud drive.

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

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 Organoids

Product	Catalog #
PneumaCult™ Airway Organoid Kit	05060

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 Technologies' cell culture supplements can be used in various stages of in vitro airway cultures, 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 airway epithelial cell research workflow. CryoStor® CS10, a serum- and animal component-free freezing medium, can be used in the expansion phase of in vitro airway culture procedures. CryoStor® CS10 contains 10% DMSO and provides a safe and protective environment for cells during the freezing, storage, and thawing processes.

Product	Size	Catalog #
CryoStor® CS10	100 mL	07930

Antibodies

Analyze cells with antibodies that are verified to work with STEMCELL Technologies' airway epithelial cell culture reagents for select applications. These primary antibodies ensure consistent results for downstream applications, including flow cytometry, immunofluorescence, and immunocytochemistry.

Target Antigen	Clone	Isotype	Catalog #
CD49f	GoH3	Rat IgG2a	60037
p63 (deltaN)	Poly6190	Rabbit IgG	60154
MUC1 (CD227)	C16A	Mouse IgG1	60155
E-Cadherin (CD324)	DECMA-1	Rat IgG1	60157

For a complete listing of available antibodies, visit **www.stemcell.com/antibodies**.

Cytokines

Activate, expand, or differentiate your cells with cytokines, chemokines, and growth factors across a variety of applications, including airway epithelial cell research. Choose reagents with >95% purity and endotoxin levels of ≤0.2 EU/µg to ensure consistency and reproducibility in your research.

Product	Catalog #	
	Non-ACF	ACF*
IL-6	78050	78148
IL-6R α	78083	-
TNF-α	78068	78157
IL-8 (CXCL8)	78084	-
Oncostatin M	78094	78199
Amphiregulin	78104	-
IL-9	78085	-
FGF-9	78161	-
GM-CSF^	78015	78140
IL-1β	78034	78143
IL-1α	78115	78219
IL-11	78025	78192
TGF-β1^	78067	-
TGF-β2	78174	-
TGF-β3	78131	78156
VEGF R2/KDR Fc	78130	-

**All ACF cytokines are human recombinant proteins produced in E. coli and are guaranteed free of animal or human components.*

^International Units (IU) data available. Visit www.stemcell.com/IU-data.

For a complete listing of cytokines available, including animal-component free (ACF) versions, please visit **www.stemcell.com/cytokines**.

Cell Dyes and Stains

Cell Counting / Viability Reagents

Cell counts are an integral part of most experiments, and are often performed to monitor cell health, viability, and proliferation rate, or to determine seeding concentrations.

Product	Size	Catalog #
Trypan Blue	100 mL	07050
DAPI	10 mg	75004

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. Stained cells can also be cryopreserved without loss of fluorescence intensity.

Product	Size	Catalog #
GloCell™ Fixable Viability Dye Red 710	100 Tests	75006.1
	5 x 100 Tests	75006
GloCell™ Fixable Viability Dye Red 780	100 Tests	75007.1
	5 x 100 Tests	75007
GloCell™ Fixable Viability Dye UV 450	100 Tests	75008.1
	5 x 100 Tests	75008
GloCell™ Fixable Viability Dye Violet 450	100 Tests	75009.1
	5 x 100 Tests	75009
GloCell™ Fixable Viability Dye Violet 510	100 Tests	75010.1
	5 x 100 Tests	75010
GloCell™ Fixable Viability Dye Violet 540	100 Tests	75011.1
	5 x 100 Tests	75011

Learn more at www.stemcell.com/GloCell.

Annexin V Dyes

Annexin V is a characteristic cell death marker that can be used to detect early apoptotic mammalian cells. The Annexin V Apoptosis Detection Kit can be used for the combined detection of early-stage cell apoptosis using Annexin V and late-stage cell apoptosis or necrosis using both Annexin V and 7-Aminoactinomycin D (7-AAD).

Product	Size	Catalog #
Annexin V	APC, 25 Tests	100-0328
	APC, 100 Tests	100-0329
	PE, 25 Tests	100-0330
	PE, 100 Tests	100-0331
	FITC, 25 Tests	100-0332
	FITC, 100 Tests	100-0333
Annexin V Binding Buffer	50 mL	100-0334
Annexin V Apoptosis Detection Kit with 7-AAD	PE, 1 Kit	100-0337
	FITC, 1 Kit	100-0338
	APC, 1 Kit	100-0339

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

1. *Human SARS-CoV-2 IgM/IgG Rapid Test Kit is for research use only and not intended for human or animal diagnostic or therapeutic use.*

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 IgM/IgG Rapid Test Kit[1]	100-0685
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
SARS-CoV-2 (Envelope Protein) Peptide Pool	25 µg/peptide	100-0666
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**.

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AIRWAY RESEARCH

PneumaCult™ Culture Media for Human Airway Epithelial Cells

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TOLL FREE PHONE 1 800 667 0322
PHONE +1 604 877 0713
INFO@STEMCELL.COM
TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT WWW.STEMCELL.COM

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