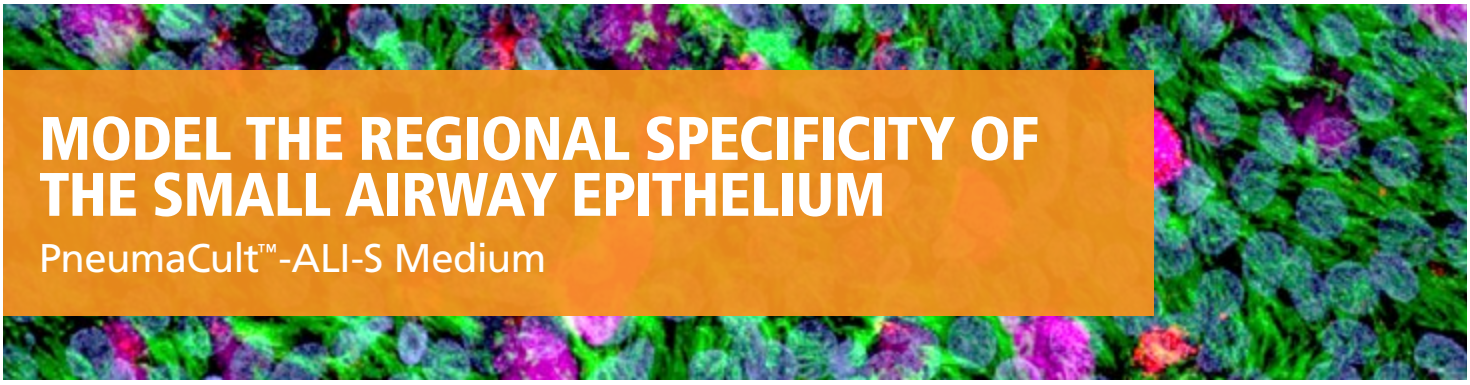

MODEL THE REGIONAL SPECIFICITY OF THE SMALL AIRWAY EPITHELIUM

PneumaCult™-ALI-S Medium

Optimized Differentiation Medium for In Vitro Small Airway Modeling

Air-liquid interface (ALI) culture is an established in vitro model that recapitulates the 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]

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, CF, and most lung cancers.[5,6] 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.[5,6] 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.[5,7] 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 bovine pituitary extract (BPE)-free differentiation medium optimized for the culture of human small airway epithelial cells (HSAEC) at the ALI. HSAEC expanded in PneumaCult™-Ex Plus and cultured in PneumaCult™-ALI-S undergo extensive mucociliary differentiation to form a thin, cuboidal epithelium that exhibits morphological and functional characteristics representative of the in vivo human small airway. Together, PneumaCult™-ALI-S and PneumaCult™-Ex Plus constitute a fully integrated serum- and BPE-free culture system for in vitro human small airway modeling. This robust and defined system is a valuable tool for basic respiratory research, toxicity studies, and drug development.

Why Use PneumaCult™-ALI-S Medium?

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

PHYSIOLOGICALLY RELEVANT. Generate in vitro small airway epithelial cell cultures that preserve morphological and functional characteristics of the in vivo human small airway.

OPTIMIZED WORKFLOW. Use with PneumaCult™-Ex Plus for a complete system for expansion, maintenance, and differentiation of small airway epithelial cells.

REPRODUCIBLE RESULTS. Reduce experimental variability and maximize reproducibility with a serum- and BPE-free formulation.

PneumaCult™-ALI-S Medium
Serum- and BPE-free medium for the culture of HSAEC at the ALI

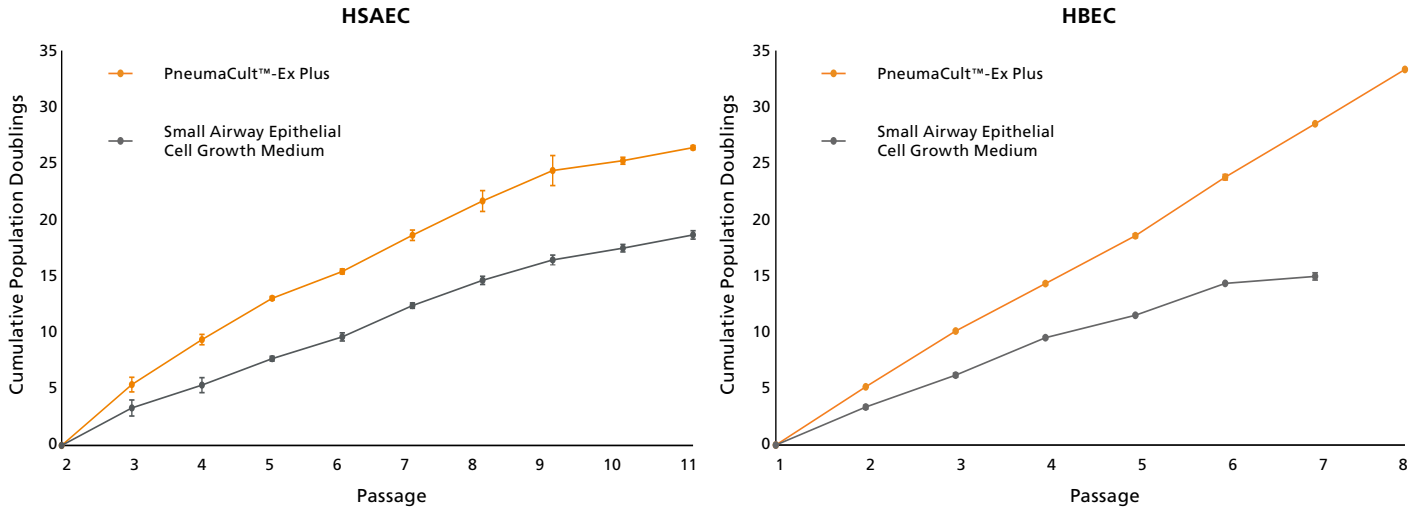

Figure 1. HSAEC and HBEC Grow at a Higher Rate During Expansion When Cultured in PneumaCult™-Ex Plus Medium

Human small airway epithelial cells (HSAEC) and human bronchial epithelial cells (HBEC) 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 HSAEC were obtained commercially at passage 2 (P2) while HBEC were obtained at P1.

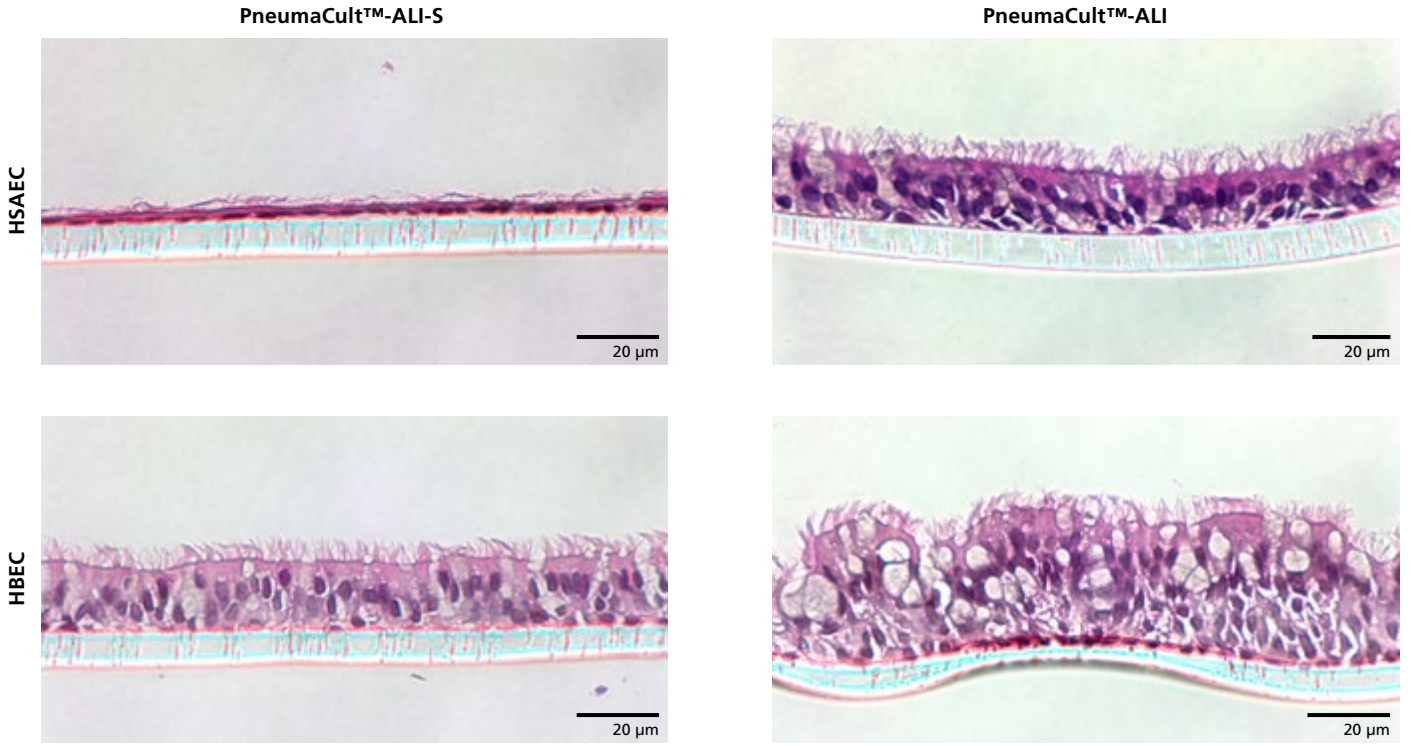

Figure 2. HSAEC Cultured at the ALI Using PneumaCult™-ALI-S Medium Differentiate to Form a Morphology Representative of the Small Airway Epithelium

Hematoxylin and eosin (H&E) staining of HSAEC and HBEC cultured in PneumaCult™-ALI-S or PneumaCult™-ALI Medium at P3, after 28 days. HSAEC differentiated at the ALI in PneumaCult™-ALI-S formed a thin, cuboidal epithelial layer representative of the in vivo small airway epithelium while HBEC differentiated in PneumaCult™-ALI formed a pseudostratified epithelium resembling the in vivo bronchial 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.

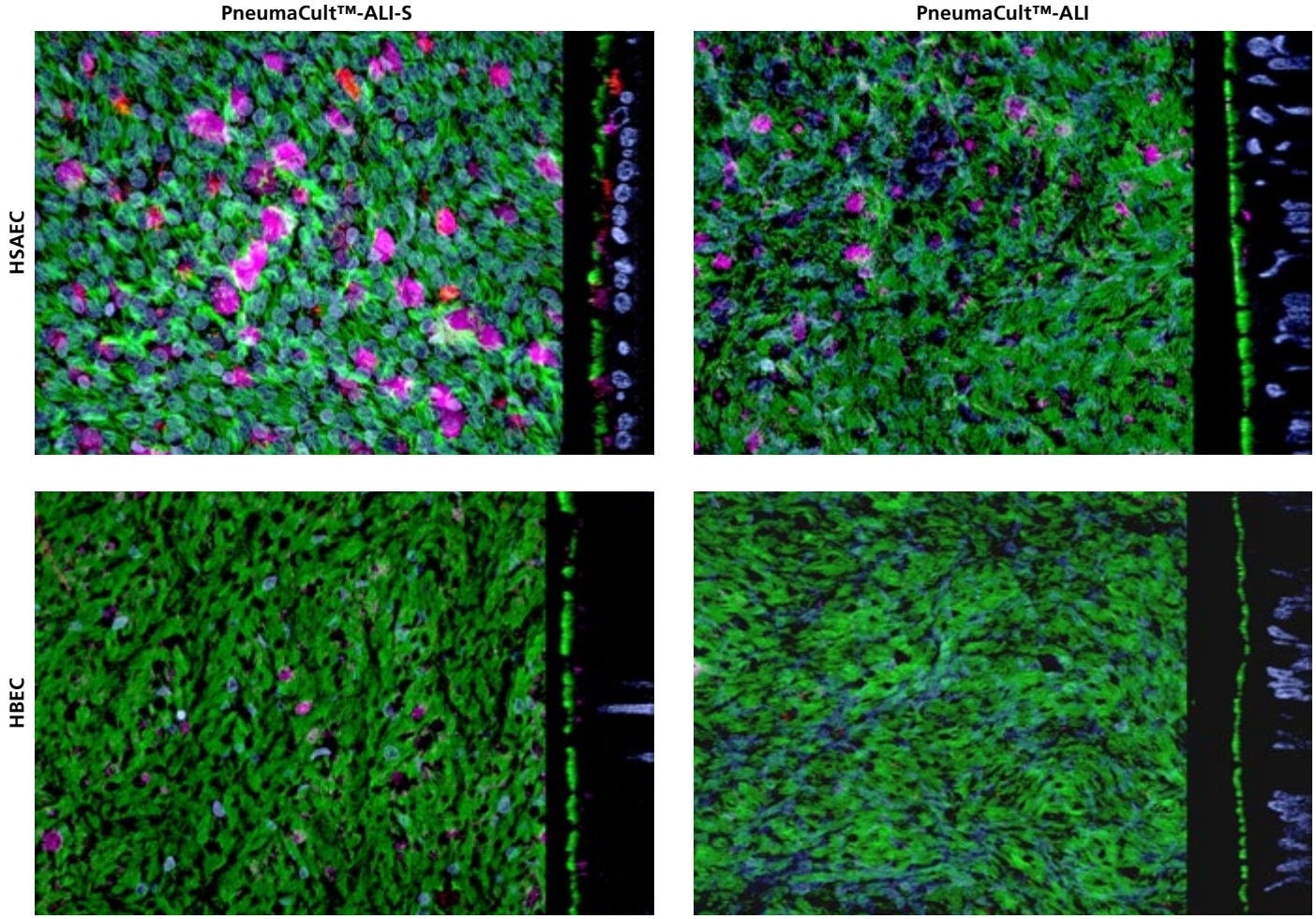

Figure 3. Small Airway Epithelium Markers Were Detected in HSAEC Cultured in PneumaCult™-ALI-S Medium

Confocal images of whole mount immunostained ALI cultures showing HSAEC and HBEC cultured in PneumaCult™-ALI-S 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 HSAEC cultured in PneumaCult™-ALI-S compared with HSAEC cultured in PneumaCult™-ALI and HBEC cultured in either PneumaCult™-ALI-S or PneumaCult™-ALI. All images were taken using a 63X objective.

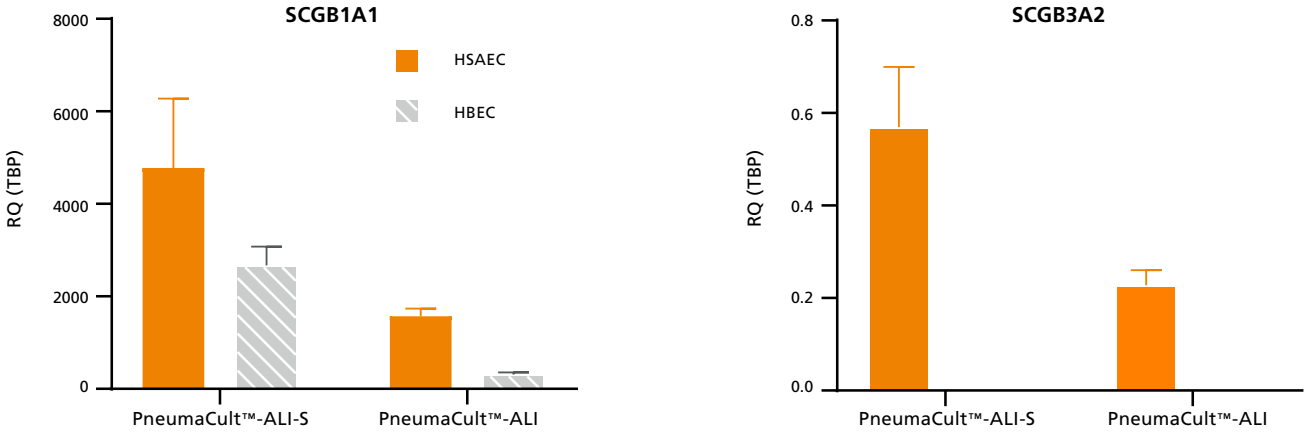

Figure 4. Relative Expression of Small Airway Epithelium Markers by qPCR Were Detected at Higher Levels in HSAEC Cultured in PneumaCult™-ALI-S Medium Compared with HSAEC Cultured in PneumaCult™-ALI

HSAEC and HBEC cultured in PneumaCult™-ALI-S or PneumaCult™-ALI Medium at P3. After 28-days of differentiation, the ALI cultures were analysed for small airway epithelium markers, SCGB1A1 and 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 HSAEC cultured in PneumaCult™-ALI-S Medium compared with HSAEC cultured in PneumaCult™-ALI and HBEC cultured in either PneumaCult™-ALI-S or PneumaCult™-ALI. Relative expression of SCGB3A2 was not detectable in HBEC cultured in either PneumaCult™-ALI or PneumaCult™-ALI-S.

Product Information

Product	Catalog #
PneumaCult™-ALI-S Medium	05050
PneumaCult™-Ex Plus Medium	05040
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
Animal Component-Free Cell Dissociation Kit	05426
Heparin Solution	07980
Hydrocortisone Stock Solution	07925

References

1. Bérubé K et al. (2010) Human primary bronchial lung cell constructs: the new respiratory models. Toxicology 278(3): 311–318.
2. Prytherch Z et al. (2011) Tissue-specific stem cell differentiation in an in vitro airway model. Macromol Biosci 11(11): 1467–1477.
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): 563–571
4. Comer D et al. (2013) Airway epithelial cell apoptosis and inflammation in COPD, smokers and nonsmokers. Eur Respir J 41(5): 1058–1067.
5. Crystal R et al. (2008) Airway epithelial cells: current concepts and challenges. Proc Am Thorac Soc 5(7): 772–777.
6. 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.
7. 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): 340–352.

SCIENTIFIC RESOURCE CENTER

Learn more about airway modeling
www.stemcell.com/airway-modeling

WALLCHART

Cellular Organization and Biology of the Respiratory System
www.stemcell.com/forms/wallchart-respiratory-system.html