Tools for Respiratory Research

PromoCell®

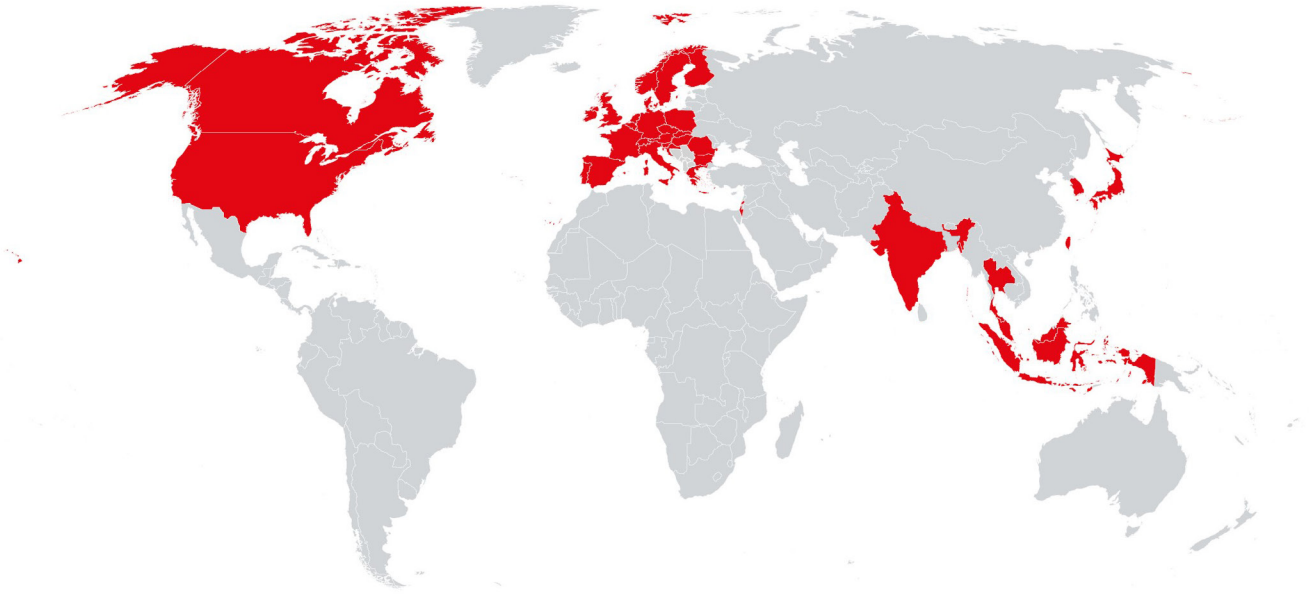
Solutions for world-class respiratory research

Human Centered Science

Who we are

At PromoCell, we help scientists do better research by offering a world-class portfolio of primary human cells, stem cells, blood cells, and optimized cell culture media. With over 30 years of expertise, we supply scientists with the tools and support they need to conduct groundbreaking research.

Each year, 600 peer-reviewed publications feature PromoCell products. We operate in over 40 countries around the world, helping scientists with their research needs—we help your science move the world forward.



Austria
Belgium
Bulgaria
Canada
Croatia
Czech Republic
Denmark
Finland
France
Germany
Greece
Hungary
India
Indonesia
Ireland
Israel
Italy
Japan
Liechtenstein
Luxembourg
Malaysia
Monaco
Netherlands
Norway
Poland
Portugal
Romania
Singapore
Slovakia
Slovenia
South Korea
Spain
Sweden
Switzerland
Taiwan
Thailand
United Kingdom
United States

Learn more about PromoCell on our **website**, or connect with us on **Facebook, YouTube, Twitter** or **LinkedIn.**

Our Commitment to Quality

Ethics and quality are at the heart of our business. We own the entire tissue collection and manufacturing process, which means we're able to provide fast and direct ethical regulatory support. All our products comply with European biomedical conventions, ensuring that human rights and donor privacy are always protected. Our ISO certifications demonstrate our commitment to quality, and our EXCiPACT™ GMP certification enables us to produce our cell culture media and reagents according to GMP standards.

- Our Quality Management System is certified according the EXCIPACT™ GMP standard
- PromoCell operates according to ISO 9001:2015 in order to consistently provide products and services that meet customer requirements as well as applicable statutory and regulatory requirements.

Our Commitment to Fighting Respiratory Diseases

Respiratory system

The respiratory tract is a complex network of organs and tissues that are vital for our survival. The respiratory network consists of the mouth, nose, sinuses, pharynx (throat), trachea, bronchial tubes, and lungs. The lungs have a highly organized hierarchical structure; they branch into lung lobes, bronchioles, and alveoli. The alveoli are in contact with capillaries, forming a complex alveolar capillary network.

Respiratory tissues are composed of various specialized cell types, including epithelial cells, fibroblasts, smooth muscle cells, and endothelial cells. These cells work together to ensure that every organ, tissue, and cell of the body is supplied with oxygen through breathing. Respiratory organs and tissues help the body extract oxygen from the air and remove carbon dioxide and other waste gases that can become toxic to the body.

Air enters the body through the respiratory system; therefore, the airways and lungs are constantly exposed to potentially harmful bacteria, viruses, fungi, and pollutants carried through the air. These inhaled irritants can cause infections and damage the lungs, leading to various respiratory diseases.

Our primary cells from the respiratory tract

Respiratory diseases are a leading cause of death and disability worldwide. Respiratory research is key to reducing the burden of lung infections, lung cancer, and asthma, which are the most common respiratory diseases. Primary cells are excellent models for studying respiratory diseases. Using our cell culture models, you can better understand the cellular and molecular mechanisms that cause lung disease, identify novel therapeutic targets, and test the efficacy and safety of new therapies for respiratory diseases. We offer an extensive portfolio of tools for basic and translational respiratory research, including various primary cell culture models.

Our respiratory models include:

- Nasal, tracheal, and bronchial airway epithelial cells
- Pulmonary smooth muscle cells
- Pulmonary fibroblasts
- Lung endothelial cells

Epithelial Cells

Human Nasal Epithelial Cells (HNEpC)*
Cat. No. C-12620

Human Tracheal Epithelial Cells (HTEpC)*
Cat. No. C-12644

Human Bronchial Epithelial Cells (HBEpC)*
Cat. No. C-12640

Human Small Airway Epithelial Cells (HSAEpC)*
Cat. No. C-12642

*Marker Expression:
Cytokeratin+

Fibroblasts

Human Pulmonary Fibroblasts (HPF)
Cat. No. C-12360

Marker Expression:
CD90+

Smooth Muscle Cells

Human Tracheal Smooth Muscle Cells (HTSMC)
Cat. No. C-12565

Human Pulmonary Artery Smooth Muscle Cells (HPASMC)
Cat. No. C-12521

Human Bronchial Smooth Muscle Cells (HBSMC)
Cat. No. C-12561

Marker Expression:
Smooth muscle α-actin+

Endothelial Cells

Human Pulmonary Artery Endothelial Cells (HPAEC)*
Cat. No. C-12241

Human Pulmonary Microvascular Endothelial Cells (HPMEC)*
Cat. No. C-12281

*Marker Expression:
CD31+
Dil-Ac-LDL uptake+

In Vitro Airway Models

In vitro airway models are becoming increasingly popular in respiratory research owing to their numerous advantages. Airways consist of a complex network of specialized cells, which are challenging to study using *in vivo* models. In contrast, 3D cultures of primary cells from donors with specific disease states provide excellent, reproducible, and physiologically relevant models for studying the *in vivo* processes underlying respiratory diseases. In addition, comparing primary cells from a donor with a specific disease to those from a healthy donor represents a straightforward and robust way to identify therapeutic targets for lung diseases.

Models of respiratory diseases

There are several respiratory diseases that vary in their etiology (i.e., smoking, air pollution, mutations, viral infections, bacterial infections), pathophysiology (e.g., inflammatory disease, effusion, neoplasia, obstructive pulmonary disease), and duration (i.e., acute or chronic). Establishing *in vivo* models that accurately mimic these complex human diseases is extremely challenging and time-consuming. In contrast, isolating primary cells from donors with specific disease states, environmental exposures (e.g., smoker versus non-smoker), and HLA types allows for the generation of versatile, functional, and physiologically relevant human airway models.

Models of human airways: Airway organoids and air-liquid interface

Human airways are complex and consist of multiple cell types that interact with each other. The spatial relationship and interplay between different cells in the airway can affect the development and progression of respiratory diseases. Airway organoids from primary human cells accurately resemble the 3D environment of human lungs. Consequently, human airway organoids are useful *in vitro* models for studying the role of the complex microenvironment and 3D architecture of airways in respiratory diseases, as well as for identifying and testing drug candidates. In contrast to cell lines used as *in vitro* airway models, air-liquid interface (ALI) culture using primary cells more accurately resembles the *in vivo* environment of human airways and enables the characterization of changes in ALI and epithelial barriers in respiratory disease.

Models of respiratory infections

Challenges associated with the establishment of animal models of human respiratory diseases are exacerbated in the case of emerging, fast-spreading, life-threatening infectious respiratory diseases, such as COVID-19. Despite extensive efforts, progress in the development of animal models of COVID-19 is limited. In contrast to animal models that require laborious and time-consuming genetic manipulations to render non-human organisms susceptible to human viruses, primary human cells can be easily infected *in vitro* to study infectious lung diseases and identify therapeutic targets.

Air-Liquid Interface Culture

Our Air-Liquid Interface (ALI) culture system enables you to generate a reproducible and physiologically relevant 3D airway model with primary human bronchial epithelial cells (HBEpCs) and functional epithelial barriers (Fig. 1). Our cell culture medium, Air-Liquid Interface Medium (ALI-Airway), is free of serum and bovine pituitary extract (BPE) and contains a low level of growth factors, helping you maintain a non-proliferating cell population over a period of at least 14 days. Cells are grown on a semipermeable porous membrane that mimics the respiratory basement membrane. The functionality of HBEpCs can be monitored through the fast development of an epithelial barrier formed by tight junctions. In addition to the ALI-Airway medium, we provide HBEpCs that are prescreened for proper barrier function in our ALI medium. We also offer HBEpC from HLA-typed donors and donors with chronic obstructive pulmonary disease (COPD) or asthma.

Key advantages of our ALI culture system:

- Minimizes lot-to-lot variation using optimized BPE-free and serum-free formulation
- Offers stable and uniform transepithelial electrical resistance (TEER) values of > 500 Ω×cm^2 for at least 14 days of culture
- Enables culturing human differentiated bronchial epithelial cells with cell viability >70%

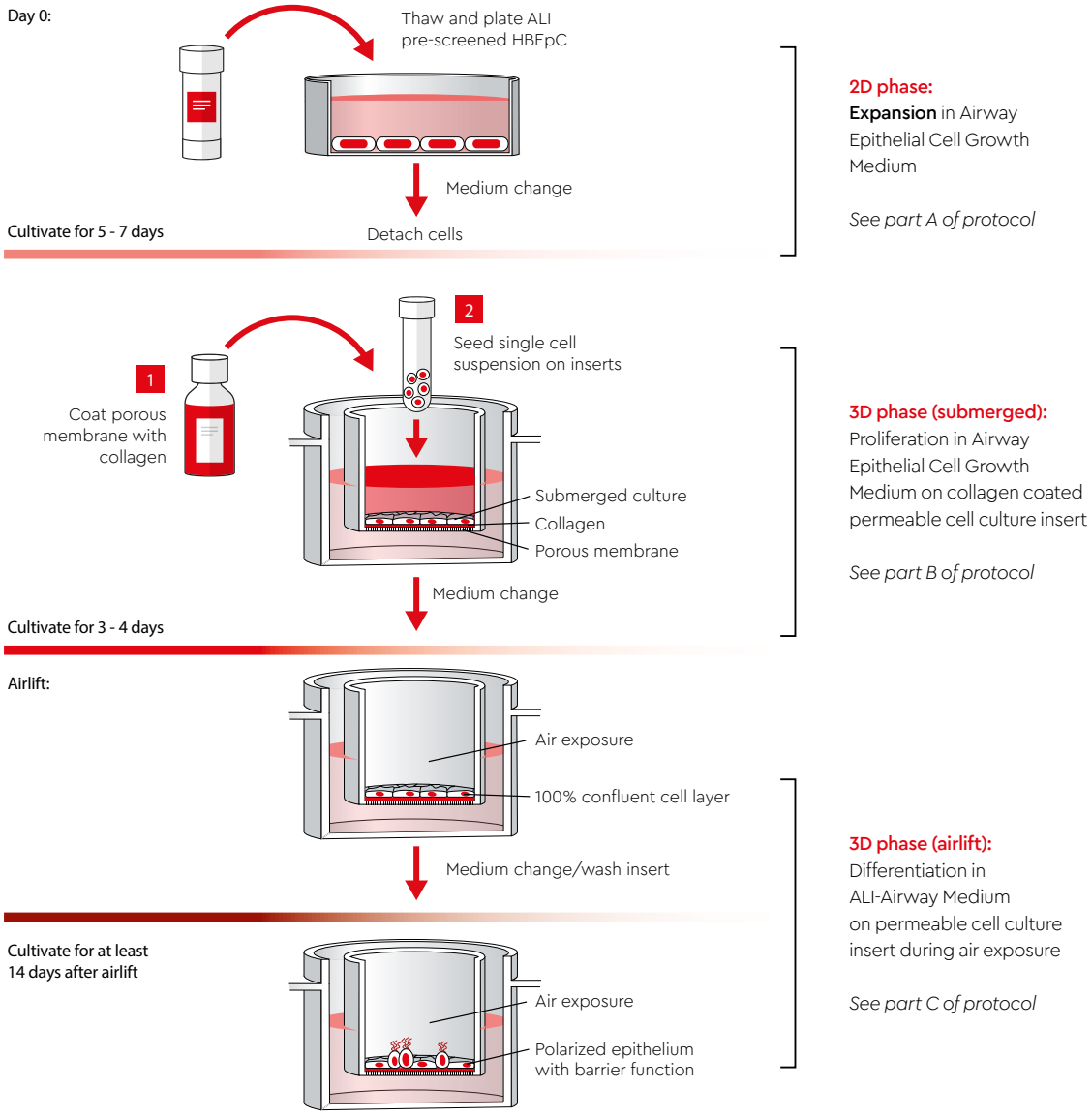


Fig. 1: ***Overview of our Air-Liquid Interface (ALI) culture system.*** *The culture workflow is divided into a 2D expansion phase and a 3D differentiation phase. After 2D expansion of ALI HBEpCs prescreened in our Airway Epithelial Cell Growth Medium, the cells are seeded into permeable cell culture insert as submerged cultures, which are allowed to grow until they reach confluence. ALI-prescreened HBEpC lots are tested for proper barrier function. Differentiation of ALI-prescreened HBEpCs is stimulated by exposure to air and culturing in ALI-Airway (C-21080). ALI-Airway consists of a basal medium and a SupplementMix (BPE- and serum-free) that enhances the barrier-forming function of HBEpCs. Collagen type I is added for optimal cell attachment because the ALI-Airway medium lacks attachment factors.*

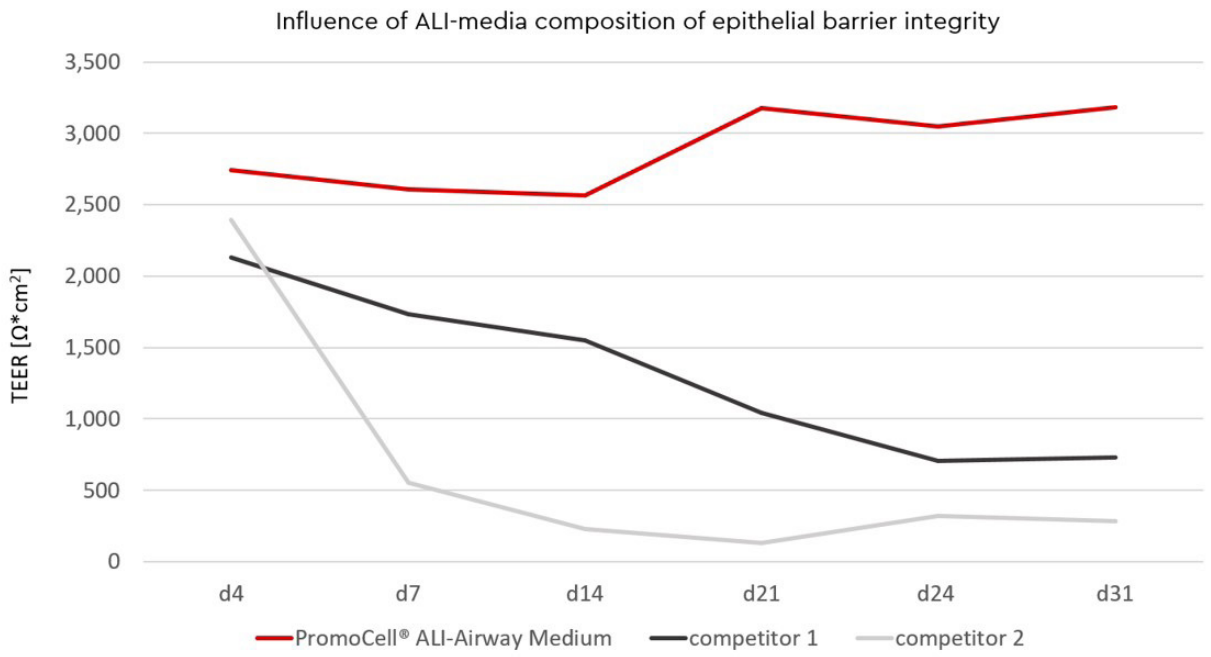


Fig. 2: ***TEER values of barrier-forming HBEpCs over a culture period of 28 days post-airlift.*** *TEER measurement was performed using EVOM® and STX® Electrode Set (World Precision Instruments®). Two different donors of prescreened HBEpCs (Donor 1 and Donor 2) were seeded on Transwell® inserts (Corning®, product number 3470). A competitor ALI medium provides lower TEER values compared to our standardized ALI system. The barrier-forming function of our ALI culture system results in an earlier increase in TEER values (1st week). This quick rise of the TEER values enables you to analyze the epithelial barrier much earlier. Our ALI-Airway medium ensures stable TEER values >500 Ω×cm² over different time points.*

Our ALI-prescreened HBEpCs maintain TEER values >500 Ω×cm² for at least 14 days of culture in our ALI culture system (Fig. 2) when you follow the instructions described in our Application Note: Air-Liquid Interface Culture System for Standardized Respiratory Research.

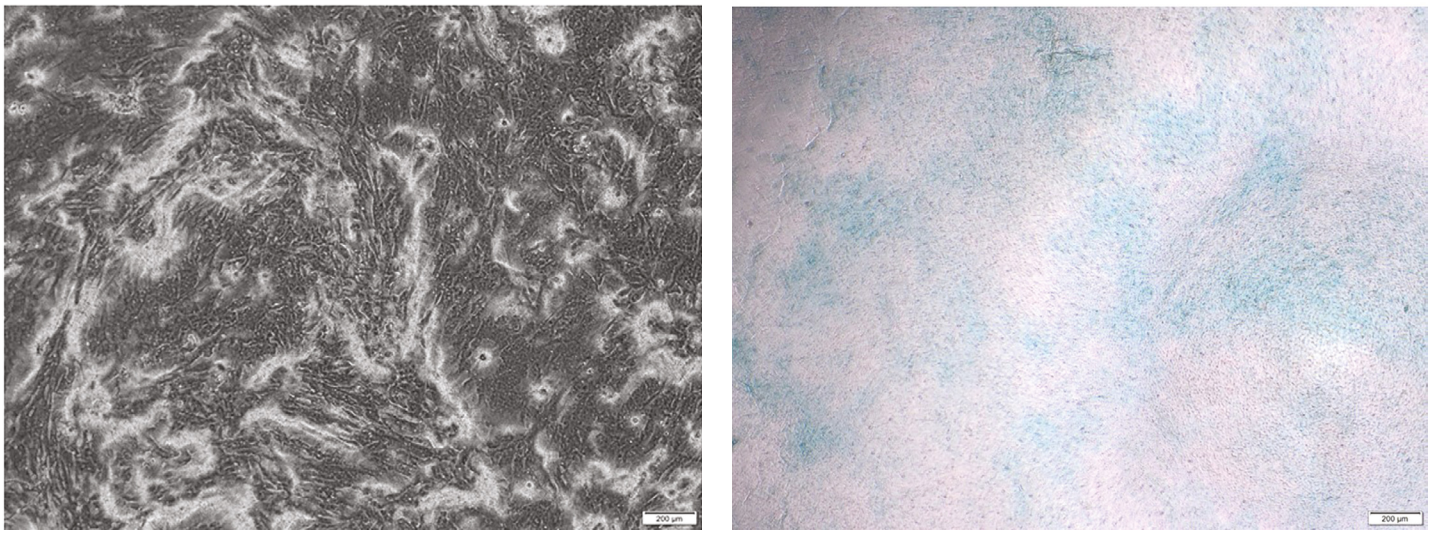


Fig. 3: ***Mucin production indicates differentiation of HBEpC cultured under air-liquid conditions. Left:*** *Microscopy of unfixed HBEpC after culturing in ALI-Airway medium for 33 days.* ***Right:*** *Alcian Blue staining of fixed cells after 33 days in culture. Alcian Blue stains sulfated and carboxylated and acid mucopolysaccharides and sulfated and carboxylated sialomucins (glycoproteins), which are indicated in blue (4x magnification, scale bar 200 µm).*

Airway Organoid Model

Our ALI-Airway medium can be used to generate 3D bronchospheres, which are organoid-like structures formed by HBEpCs when cultured in basal membrane extract (BME). Bronchospheres can be used for high-throughput drug screening. Fully differentiated organoids have a cell-free center lumen surrounded by a polarized epithelial cell layer (Fig. 3). Depending on their polarity along the apical-basal axis, multiciliated cells can face the apical membrane or the lumen of the airway organoid. Exposure of HBEpCs to the Rho-associated protein kinase inhibitor (ROCKi) Y-27632 increases their colony-forming efficiency by around 20%.

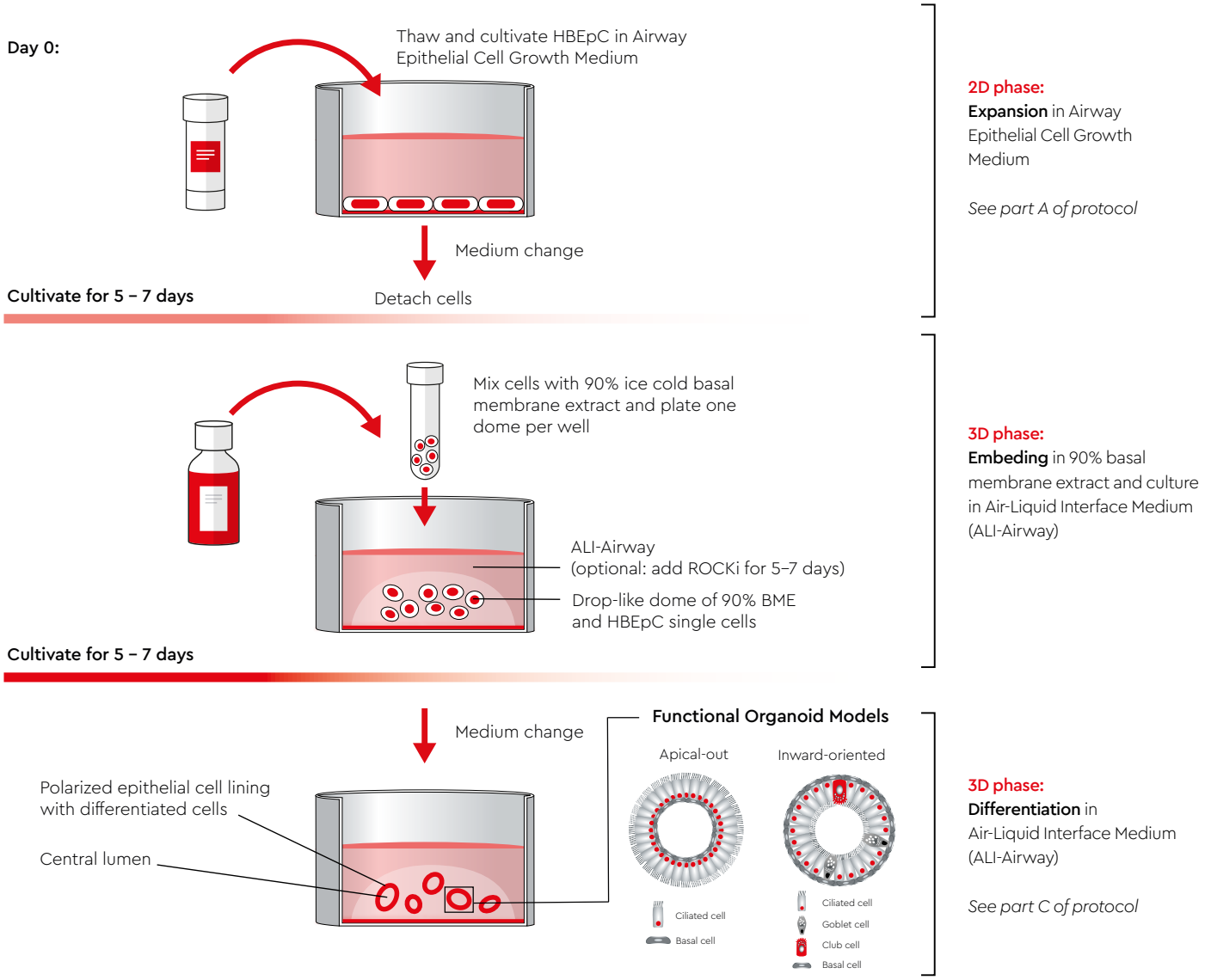


Fig. 3: Overview of our airway organoid model. *HBEpCs are expanded in 2D cell culture and re-seeded in 90% ice-cold BME drops (domes; one drop per well) in a multiwell plate. Domes are covered with ALI-Airway medium and cultured for 5–7 days. Exposure to 10 µM ROCKi Y-27632 can increase the colony-forming efficiency of HBEpCs. Differentiation of the organoids takes approximately 2 weeks of culture in fresh ALI-Airway medium. At this stage, the lumen of the 3D bronchospheres becomes visible, and ciliated cells populate the polarized epithelial cell lining facing the apical membrane ('apical-out') or the lumen ('inward-oriented') of the airway organoid. The differentiation process is completed after 4 weeks of culture in ALI-Airway medium.*

Bronchospheres cultured in our ALI-Airway medium show the first signs of differentiation after 2 weeks of culture when ciliated cells become visible. Ciliated cells maintain their high self-renewal potential for over 4 weeks. The second stage of differentiation is the formation of the airway organoid lumen, which can be visualized by microscopy (Fig. 4). Organoids with apical-out ciliated cells tend to rotate in the matrix because of synchronous ciliary beating. Differentiation is completed after 4 weeks of 3D culture.

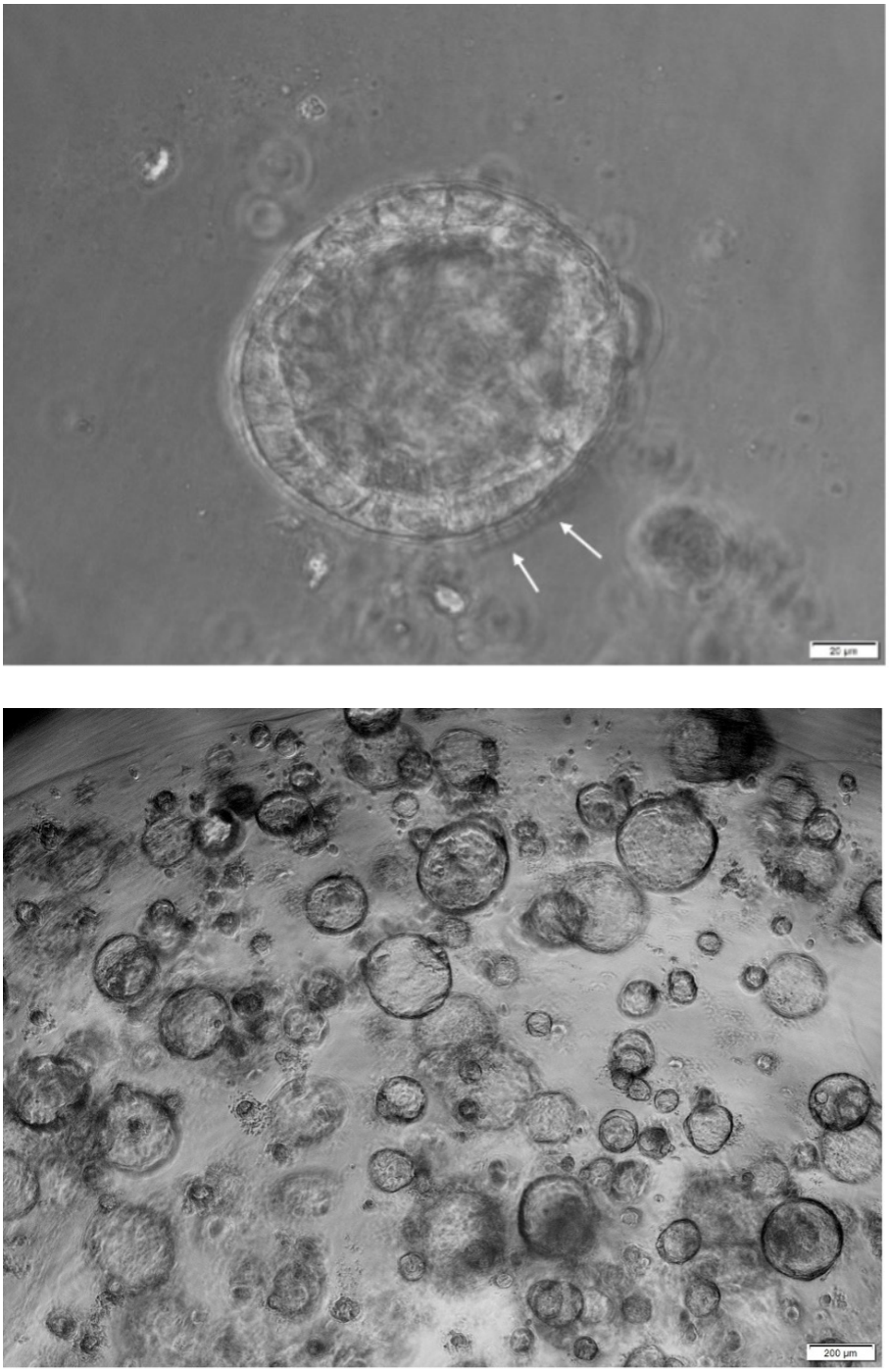


Fig. 4: Differentiated airway organoids (3D bronchospheres). Top: Bright-field microscopy images showing differentiation of HBEpC after 16 days of 3D culture in ALI-Airway medium. At this stage, the polarized epithelial cell lining of the organoids and the inner central lumen become visible. Arrows indicate apical ciliated cells. Scale bar = 20 µm. **Bottom:** Long-term culture of airway organoids, which maintain their self-renewal potential. HBEpCs were seeded in multiwell plates (10.000 cells/well). HBEpCs were cultured in ALI-Airway medium containing 10 µM Y-27632 and 90% BME. Organoids were cultured for over 38 days in ALI-Airway medium. Note that airway organoids maintain their central lumen and polarization after long-term culture. Scale bar = 200 µm.

Models for Chronic Respiratory Diseases

Respiratory diseases represent a significant healthcare and economic burden and are a leading cause of death. Common diseases of the respiratory system include chronic obstructive pulmonary disease (COPD), asthma, lung cancer, multidrug-resistant bacterial infections (e.g., tuberculosis), and infectious diseases (e.g., influenza and SARS). Our wide range of primary cells from patient donors can help you identify new therapeutic targets and evaluate the efficacy and safety of potential drug candidates for the treatment of acute and chronic respiratory diseases.

What we offer

Donors with COPD Type I–IV	■ Bronchial Epithelial Cells (HBEpC) ■ Small Airway Epithelial Cells (HSAEpC) ■ Bronchial Smooth Muscle Cells (HBSMC) ■ Pulmonary Fibroblasts (HPF)
Donors with asthma	■ Bronchial Epithelial Cells (HBEpC) ■ Small Airway Epithelial Cells (HSAEpC) ■ Bronchial Smooth Muscle Cells (HBSMC) ■ Pulmonary Fibroblasts (HPF)
Donors tested negative for COVID-19	■ Nasal Epithelial Cells (HNEpC) ■ Pulmonary Microvascular Endothelial Cells (HPMEC)

Our primary cells allow for the establishment of respiratory disease models with different smoker statuses (i.e., never-smoker, non-smoker, and smoker) and HLA types. Our streamlined tissue procurement with established partners enables us to source tissues on a regular basis and provide you with a wide variety of cell models and lot sizes that align with your experimental needs.

Advantages of our primary cells from patient donors

- Verified cell identity through cell marker expression and morphology
- Verified cell growth performance and cell morphology for several passages for each lot
- Wide variety of model characteristics and lot sizes

All cell types from patient donors can be cultured in our cell culture media to ensure optimal growth performance for your experiments.

Our Tools for Respiratory Research

Human Primary Cells

Cell type	Description	Cat. nº.	Marker	Recommended culture media*
Epithelial Cells	Human Nasal Epithelial Cells (HNEpC)	C-12620	Cytokeratin$^+$	C-21060
	Human Tracheal Epithelial Cells (HTEpC)	C-12644	Cytokeratin$^+$	C-21060
	Human Bronchial Epithelial Cells (HBEpC)	C-12640	Cytokeratin$^+$	C-21060
	Human Small Airway Epithelial Cells (HSAEpC)	C-12642	Cytokeratin$^+$	C-21070
Fibroblasts	Human Pulmonary Fibroblasts (HPF)	C-12360	CD90$^+$	C-23020
Endothelial Cells	Human Pulmonary Artery Endothelial Cells (HPAEC)	C-12241	CD31$^+$ Dil-Ac-LDL uptake$^+$	C-22010/C-22011
	Human Pulmonary Microvascular Endothelial Cells (HPMEC)	C-12281	CD31$^+$ Dil-Ac-LDL uptake$^+$	C-22020/C-22022
Smooth Muscle Cells	Human Pulmonary Artery Smooth Muscle Cells (HPASMC)	C-12521	Smooth muscle α-actin$^+$ CD31$^-$	C-22062
	Human Tracheal Smooth Muscle Cells (HTSMC)	C-12565	Smooth muscle α-actin$^+$ CD31$^-$	C-22062
	Human Bronchial Smooth Muscle Cells (HBSMC)	C-12561	Smooth muscle α-actin$^+$ CD31$^-$	C-22062

*The catalog numbers in this table are for media in ready-to-use packaging.

Cell Culture Media for Expansion and Differentiation

Cell type	Product	Size	Catalog Number
Epithelial Cells	Airway Epithelial Cell Growth Medium	500 ml	Ready-to-use C-21060 / Kit C-21160
	Small Airway Epithelial Cell Growth Medium	500 ml	Ready-to-use C-21070 / Kit C-21170
	Air-Liquid Interface Medium (ALI-Airway)	500 ml	C-21080
Fibroblasts	Fibroblast Growth Medium 2	500 ml	Ready-to-use C-23020 / Kit C-23120
Endothelial Cells	Endothelial Cell Growth Medium	500 ml	Ready-to-use C-22010 / Kit C-22110
	Endothelial Cell Growth Medium 2	500 ml	Ready-to-use C-22011 / Kit C-22111
	Endothelial Cell Growth Medium MV	500 ml	Ready-to-use C-22020 / Kit C-22120
	Endothelial Cell Growth Medium MV 2	500 ml	Ready-to-use C-22022 / Kit C-22121
Smooth Muscle Cells	Smooth Muscle Cell Growth Medium 2	500 ml	Ready-to-use C-22062 / Kit C-22162

Reagents for Dissociation and Cryopreservation

Product	Size	Catalog Number
DetachKit	30 ml	C-41200
	125 ml	C-41210
	250 ml	C-41220
Cryo-SFM	30 ml	C-29910
	125 ml	C-29912

References

1. Pezzulo, A.A., et al., The air-liquid interface and use of primary cell cultures are important to recapitulate the transcriptional profile of *in vivo* airway epithelia. American Journal of Physiology-Lung Cellular and Molecular Physiology, 2010;300(1):L25-L31.
2. Zhou, J., et al., Differentiated human airway organoids to assess infectivity of emerging influenza virus. Proceedings of the National Academy of Sciences, 2018;115(26):6822–6827.
3. Narumiya, S., Ishizaki, T., and Ufhata, M., Use and properties of ROCK-specific inhibitor Y-27632. Methods in Enzymology, 2000;325:273–284.
4. Barkauskas, C.E., et al., Lung organoids: current uses and future promise. Development, 2017;144(6):986–997.
5. Di Cicco, M., et al., Structural and functional development in airways throughout childhood: Children are not small adults. Pediatric Pulmonology, 2021;56(1):240–251.
6. Santacroce, L., et al., The human respiratory system and its microbiome at a glimpse. Biology, 2020;9(10):318.
7. Miller, A.J. and Spence, J.R. *In vitro* models to study human lung development, disease and homeostasis. Physiology (Bethesda), 2017;32(3):246–260.
8. Li, H., et al., Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. Signal Transduction and Targeted Therapy, 2020;5(1).
9. Ishikawa, S., Ishimori, K. and Ito, S, A 3D epithelial–mesenchymal co-culture model of human bronchial tissue recapitulates multiple features of airway tissue remodeling by TGF-β1 treatment. Respiratory Research, 2017;18(195).
10. Baldassi, D., Gabold, B. and Merkel, O., Air-liquid interface cultures of the healthy and diseased human respiratory tract: promises, challenges and future directions. Advanced Nanobiomed Research. 2021;1(6):2000111.
11. Han, Y., et al., Human organoid models to study SARS-CoV-2 infection. Nat Methods, 2022;19:418–428 (2022).
12. Heinen, N., et al., *In vitro* lung models and their application to study SARS-CoV-2 pathogenesis and disease. Viruses, 2021;13(5):792.

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

info@promocell.com
www.promocell.com

USA/Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 – 90 93 32 (ligne verte)
Téléfax: 0800 – 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 96 03 33 (toll free)
Fax: 0800 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

M-6631 | 09/2023