Isolation and expansion of primary airway epithelial cells

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Application note

Background

The burden of chronic respiratory diseases is increasing, with chronic obstructive pulmonary disease (COPD), asthma, and cystic fibrosis affecting millions worldwide.[1] Understanding the molecular mechanisms underlying respiratory diseases requires the use of laboratory models that recapitulate human airway biology.

Types and applications of airway epithelial cells

The respiratory tract contains various types of epithelial cell populations. These include human nasal epithelial cells (HNEpC) in the nasal mucosa, human tracheal epithelial cells (HTEpC) in the tracheal surface epithelium, and human bronchial epithelial cells (HBEpC) in the epithelium of the bronchial tubes.

HNEpC serve as models for studying respiratory infections, including viral pathogens such as SARS-CoV-2, and for evaluating therapeutics administered nasally.[2] HTEpC can be used to study upper airway physiology and diseases.[3] Primary HBEpC have emerged as the gold standard for *in vitro* respiratory research and the development of drugs for respiratory diseases. They can be used to investigate diseases such as COPD, asthma, and cystic fibrosis.[4] HBEpC can also be used to study the pulmonary effects of environmental exposures such as cigarette smoke and air pollution.[5]

Physiological relevance of primary epithelial cultures

Compared to immortalized cell lines, primary airway epithelial cells better recapitulate the cellular characteristics of the human lung. During *in vitro* differentiation, basal cells within primary cultures proliferate and differentiate to generate a bronchial epithelium comprising ciliated cells, goblet cells, and club cells.[6] This differentiation process resembles airway repair mechanisms, providing researchers with a model for studying epithelial regeneration after injury. In addition, the genetic diversity in primary cells enables investigation of patient-specific responses. This includes the influence of HLA polymorphisms and comorbidities on disease susceptibility and therapeutic outcomes.[7]

Challenges of working with airway epithelial cells

Early serum-free culture systems for airway epithelial cells, developed in the 1980s, relied on supplementation with bovine pituitary extract (BPE). These systems had poor growth rates and limited proliferative capacity.[8,9] Subsequent systems incorporated fibroblast feeder layers or extracellular matrix components to support long-term culture maintenance.[10]

New approaches that entail the use of conditionally reprogrammed cell technology have demonstrated enhanced proliferative potential through the supplementation of small molecules. These molecules include mTOR, SMAD, and ROCK pathway inhibitors.[11–14] However, these systems lack standardization. Inconsistent requirements for extracellular matrix coating, feeder cell support, or undefined serum components limit experimental reproducibility across laboratories.[11–14]

Isolation of primary airway epithelial cells is labor-intensive, and extensive cell characterization is necessary to ensure epithelial identity and functionality.

Additionally, the use of animal-derived components in cell culture media for airway epithelial cells poses translation barriers. This introduces experimental variables that may confound research findings.[15,16]

Long-term expansion of airway epithelial cells with differentiation potential is challenging because they lose their biological characteristics with increasing numbers of passages.[12] The limited lifespan of cells *in vitro* reflects Hayflick's observation that diploid cells can divide approximately 50 (±10) times until they end their replicative status caused by gradual telomere shortening.[17] Isolated lung basal cells show the same trend in decline of regenerative potential as seen in aging or airway disease progression. State-of-the-art culture media should, therefore, maintain the physiologically relevant cell characteristics found in living tissue: a mixture of cell subpopulations in an ongoing asynchronous homeostasis.[18] Airway culture media that exceed the Hayflick limit of cell expansion may lead to a broad inflammatory phenotype and altered morphology combined with a loss of contact inhibition, which could interfere with experimental outcomes.[18] Media-dependent effects on airway subpopulation profile, cytokine release, or vessel surface-dependent ECM deposition need to be considered when interpreting results of cell culture models.[6,18]

These limitations underscore the need for improved, standardized protocols. Such protocols should reliably support the isolation, expansion, and maintenance of primary airway epithelial cells while preserving their physiological characteristics and research utility.

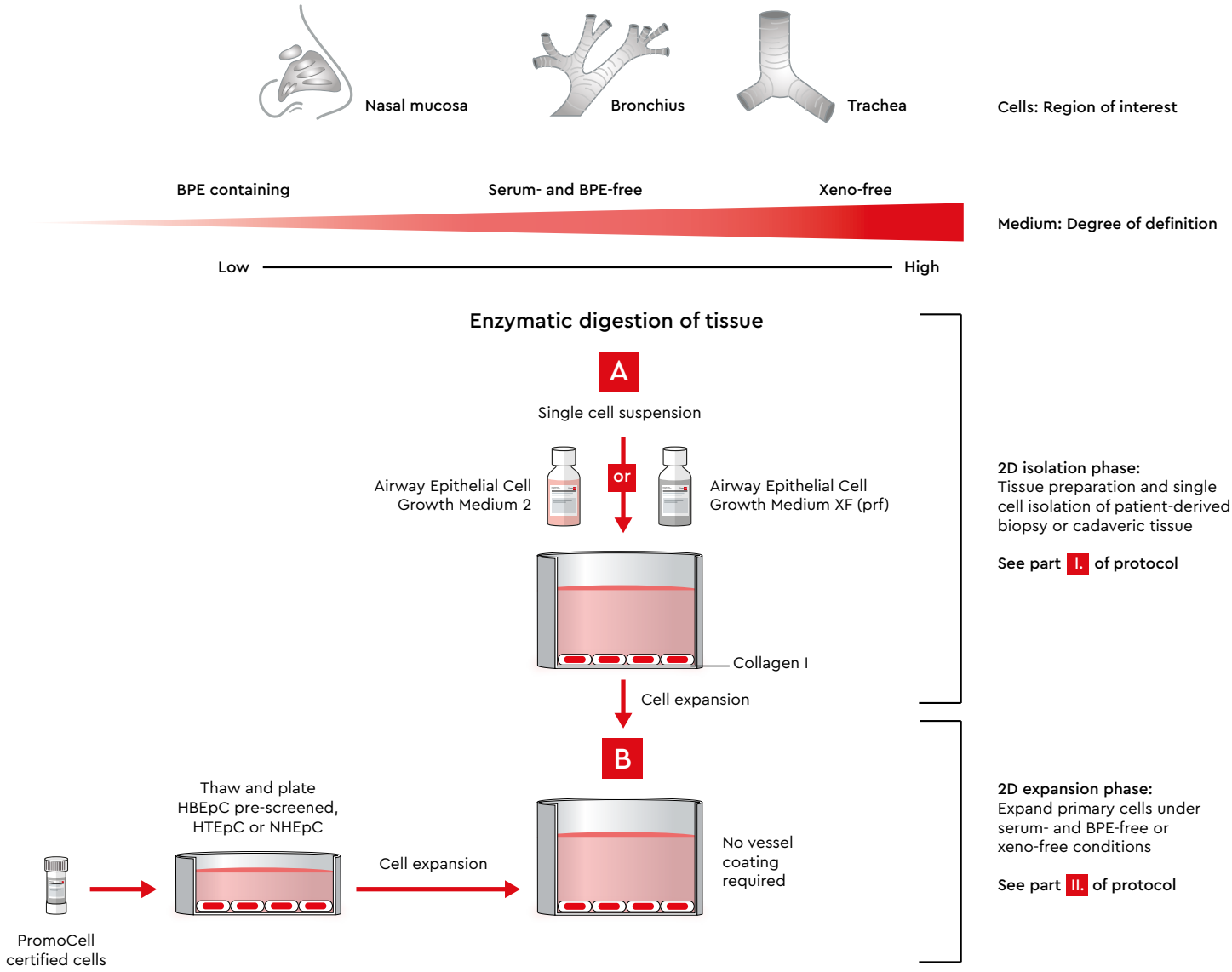

Fig. 1: Workflow for isolation and expansion of patient-derived airway epithelial cells. *Following enzymatic tissue digestion and single-cell suspension preparation, cells undergo a two-phase culture process:* ***(A)*** *2D isolation phase using collagen I-coated vessels with medium selection based on desired cell culture medium definition (serum-and BPE-free to xeno-free conditions), and* ***(B)*** *2D expansion phase where primary cells are expanded under serum- and BPE-free or xeno-free conditions without vessel coating. Alternatively, you can thaw and expand our certified airway epithelial cells in 2D expansion phase.*

Protocol

Protocol for isolation and expansion of HBEpC, HTEpC, and HNEpC

This chapter describes the isolation and expansion of primary airway epithelial cells derived from healthy human tissue of nasal mucosa, main bronchi/mucosa, or trachea/mucosa in either Airway Epithelial Cell Growth Medium 2 or Airway Epithelial Cell Growth Medium XF (prf). As a tissue source, patient-derived samples (≤24 h) or fresh cadaveric tissue (≤2 days) can be used following established ethical standards in your lab.

I. Isolation protocol

Materials:

- Airway Epithelial Cell Growth Medium 2 (C-21040) or Airway Epithelial Cell Growth Medium XF (prf) (C-21050)
- Tissue culture treated cell culture vessel, precoated with collagen (e.g., BioCoat® Collagen I Cellware, product number 354551 Corning® Incorp.)
- Tissue culture treated cell culture vessel without coating (e.g., Nunclon™ Delta Surface Nunc Dish, product number 168381 Thermo Fisher Scientific)
- Physiological buffer (Dulbecco's PBS w/o $Ca^{++}Mg^{++}$ C-40230/C-40232, HEPES Buffered Saline Solution C-40000 or similar)
- Protease XIV solution
- Gentamicin sulfate solution at a final concentration of 50 µg/ml (optional)
- 70% EtOH
- Scalpel, centrifuge tube, filter

1

Prepare airway growth medium

Thaw the SupplementMix at 15–25°C. Under aseptic conditions, mix the supplement solution by carefully pipetting up and down. Then transfer the entire contents of the supplement to the 500 ml of growth medium. The medium contains light-sensitive components and should be protected from light. Our media do not contain antibiotics. For primary cell isolation from human tissue, the use of antibiotics is optional. If you wish to add antibiotics, we recommend using a concentration of 50 µg/ml gentamicin sulfate in the final medium.

2

Adjust the medium to room temperature

Calculate the amount of airway growth medium (serum- and BPE-free or xeno-free formulation) needed for isolation. Prewarm only an aliquot of the complete medium protected from light, and keep the remaining medium refrigerated at 4–8°C.

3

Rinse the tissue with physiological buffer

4

Prepare the tissue and cut it into small pieces

Place the tissue in a sterile dish under a laminar air flow cabinet and remove any connective tissue, cartilage, fat, or blood vessels.

Depending on your tissue:

- If you use nasal mucosa for HNEpC isolation, cut the remaining tissue into small pieces and wash them with buffer.
- For the isolation of bronchial or tracheal epithelial cells, isolate the inner lining of the lumen (lamina propria) using forceps, wash the tissue with buffer, and then cut it into small pieces.

5

Digest the tissue to generate a single-cell solution

Place the washed tissue pieces into a fresh container, add Protease XIV solution, and incubate at 37°C.

6

Collect the single-cell solution

Filter the cell suspension and collect the cells by centrifugation. Resuspend the cell pellet in Airway Epithelial Cell Growth Medium 2 or Airway Epithelial Cell Growth Medium XF (prf) and count the cells using your standard method.

7

Plate the cells

For serum- and BPE-free media as well as for xeno-free media, collagen-coated vessels are required for the initial seeding. For further expansion, ordinary cell culture treated plastic without extracellular matrix (ECM) can be used. We recommend collagen I-coated plates from Corning® (BioCoat®). Plate the cells at a seeding density of 10,000 cells per cm^2 in serum- and BPE-free or xeno-free medium. Calculate the number of culture vessels. Plate the cells in growth medium (~180 µl medium per cm^2). Incubate the culture vessel in a humidified incubator (37°C, 5% CO_2).

II. Culture protocol

Materials:

- Airway Epithelial Cell Growth Medium 2 (C-21040) or Airway Epithelial Cell Growth Medium XF (prf) (C-21050)
- Culture vessel (e.g., Nunclon™ tissue culture dish product number 168381, Thermo Fisher Scientific)
- DetachKit (C-41210; 125 ml) containing the following products: HEPES Buffered Saline Solution, 0.04% Trypsin/0.03% EDTA, and Trypsin Neutralization Solution (0.05% Trypsin inhibitor in 0.1% BSA)
- Optional: Physiological buffer (Dulbecco's PBS C-40230/C-40232, HEPES BSS C-40000 or similar)

1

Let the cells grow

Change the medium every 2–3 days.

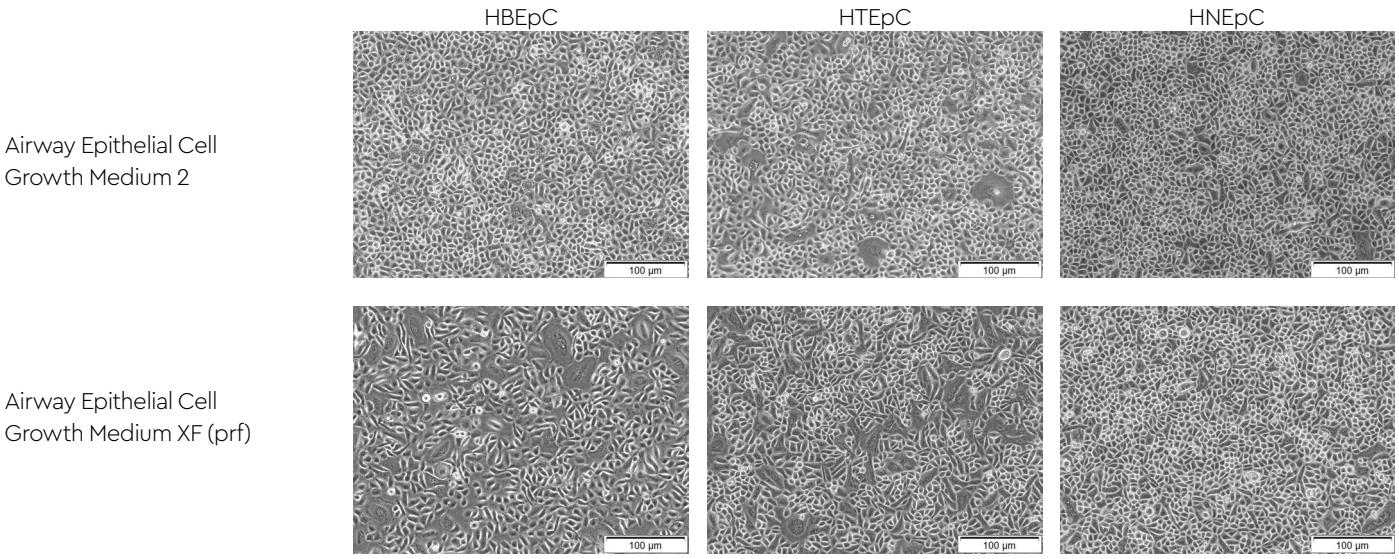

Fig. 2: Typical small and homogenous cobblestone morphology of different airway epithelial cell types grown in our serum- and BPE-free or xeno-free airway growth media. *Cells were isolated from healthy human donor tissue (main bronchi, trachea, or nasal mucosa) and seeded on collagen I-coated plates at 10,000 cells per cm^2 in serum- and BPE-free Airway Epithelial Cell Growth Medium 2* ***(upper panel)*** *or Airway Epithelial Cell Growth Medium XF (prf)* ***(lower panel)****. Cells grow tightly packed as small, almost round cells. Tissue-derived cell preparations do not show contamination of fibroblasts, which can occur in undefined medium containing serum or BPE. Microscopy photos were taken at the time of first passage (10x magnification, scale bar = 100 µm).*

2

Detach the cells when reaching ≥70% confluence

Once the cells have reached ≥70% confluence, they can be passaged. Carefully aspirate the culture medium. Add 0.15 ml µl HEPES BSS per cm^2 of vessel surface to wash the cells, and agitate the vessel carefully for 15 seconds. Aspirate the HEPES BSS (optional Dulbecco's PBS w/o Ca^{++}/Mg^{++}) and cover the cells with 50 µl Trypsin/EDTA Solution per cm^2. Transfer the vessel to an incubator for 4 minutes.
Note: We recommend detaching the cells at 37°C for 4 minutes. Close the vessel and examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells. Due to their high amount of adhesion proteins, epithelial cells tend to be very sticky on cell culture plastics. Do not over-trypsinize the cells, and gently wash the cells from the plastic.

3

Harvest the detached cells

When cells are detached, add 50 µl Trypsin Neutralization Solution per cm^2 and gently agitate the cell culture vessel. Resuspend the cells and transfer the cell suspension into a centrifuge tube containing an appropriate volume of your chosen airway growth medium. Rinse the culture vessel with HEPES BSS (optional Dulbecco's PBS w/o Ca^{++}/Mg^{++}) and pool the cell suspension into the centrifuge tube. Place the culture vessel under a microscope and verify that all the airway epithelial cells have been harvested. Centrifuge the cell suspension at 300 x g for 3 minutes.

4

Discard the supernatant and resuspend the cell pellet carefully by pipetting up and down in an appropriate volume of airway growth medium.

5

Count the cells using your standard method

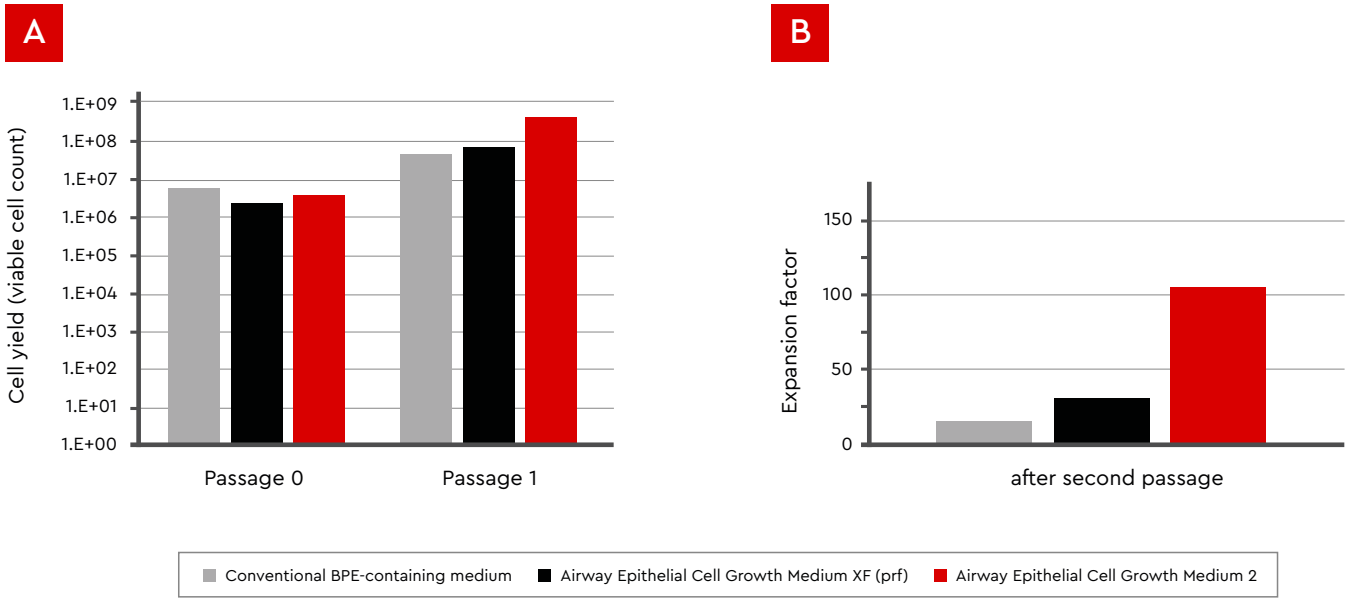

Fig. 3: Increased cell yield of isolated HNEpC in physiologically relevant media without serum or BPE. *Primary HNEpC were isolated from fresh healthy human tissue (nasal mucosa), and the enzymatically generated single-cell suspension was seeded on collagen I-coated tissue culture treated plate (Corning®) at 10,000 cells per cm^2 in three different airway growth media. The medium was changed every 2–3 days until the cells reached 70%–90% confluence. Cells were detached using Trypsin/EDTA, and the number of viable cells was analyzed using Muse® Cell Analyzer (Cytek®). For further expansion, cells were reseeded at 5,000 cells per cm^2 on tissue culture treated vessels without the need for ECM coating.* ***A)*** *Cell yield (cell counts) of viable HNEpC in passage 0 and passage 1 grown in three different airway growth media.* ***B)*** *Comparison of the mean expansion factor of viable proliferating HNEpC in three different airway growth media. Mean values of three isolated HNEpC donors were calculated using cell counts from the first and second passages.*

6

Reseed the cells in a culture vessel

No vessel coating with ECM is required. Use a seeding density of 5,000 cells per cm^2.

7

Change the medium every two or three days

Use airway epithelial growth medium for serial expansion of the cells. We recommend using the cells in early passage for experiments. Our primary airway epithelial cells can be cultivated for at least 15 population doublings (see Figure 4).

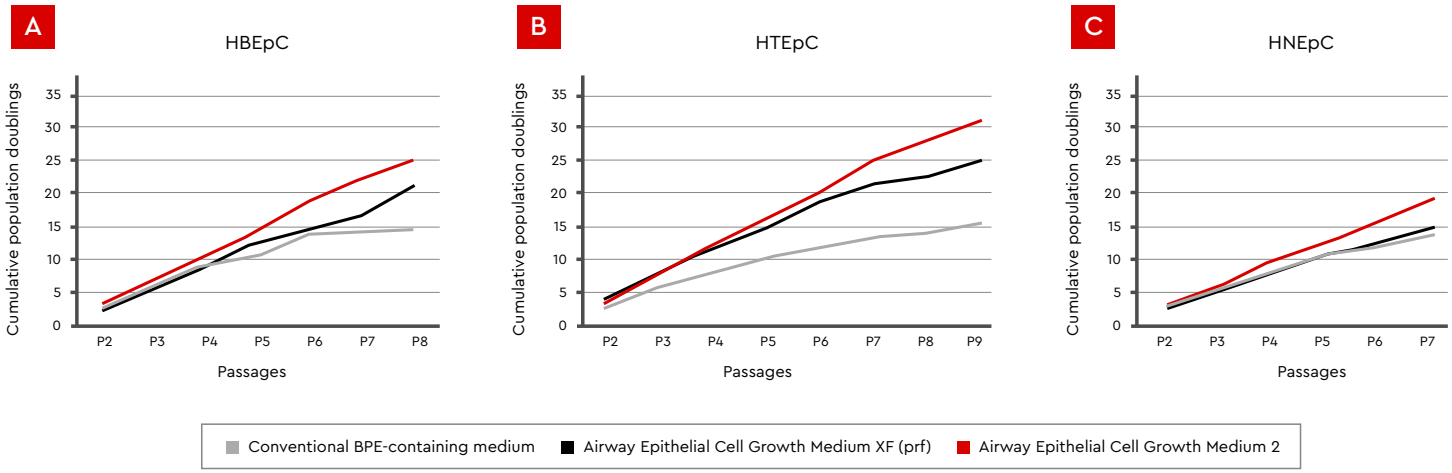

Fig. 4: Highly proliferative airway epithelial cells in our airway media. *Cryopreserved airway epithelial cells were seeded in our three different airway growth media at 5,000 cells per cm^2 on tissue culture treated plastic (Falcon®). Samples were collected using Trypsin/EDTA when the cells reached 50%–90% confluence and then reseeded at a density of 5,000 cells per cm^2. Cells were cultivated for at least 15 population doublings.* ***A)*** *Growth curve of primary human HBEpC.* ***B)*** *Growth curve of primary HTEpC.* ***C)*** *Growth curve of primary HNEpC.*

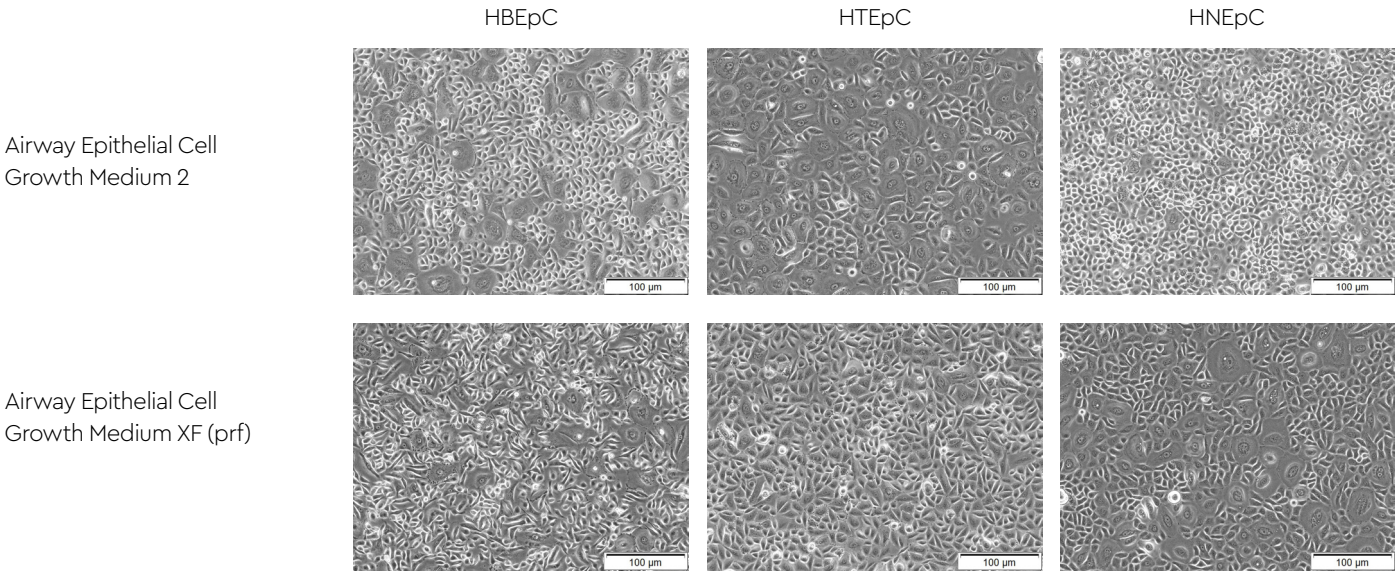

Fig. 5: Maintenance of typical cobblestone morphology of highly proliferative airway epithelial cells. *Cryopreserved airway epithelial cells were seeded in two different airway growth media at 5,000 cells per cm^2 on tissue culture treated plastic (Falcon®). Samples were collected using Trypsin/EDTA when the cells reached 50%–90% confluence and then reseeded at a density of 5,000 cells per cm^2. Cells in higher passages cultivated in the Airway Epithelial Cell Growth Medium XF (prf) tend to have more cells with increased cell size compared to those cultured in serum- and BPE-free Airway Epithelial Cell Growth Medium 2.* ***Left side:*** *Microscopy of primary HBEpC in passage 8.* ***Middle:*** *Microscopy of primary HTEpC in passage 7.* ***Right side:*** *Microscopy of primary HNEpC in passage 7 (10x magnification; scale bar = 100 µm).*

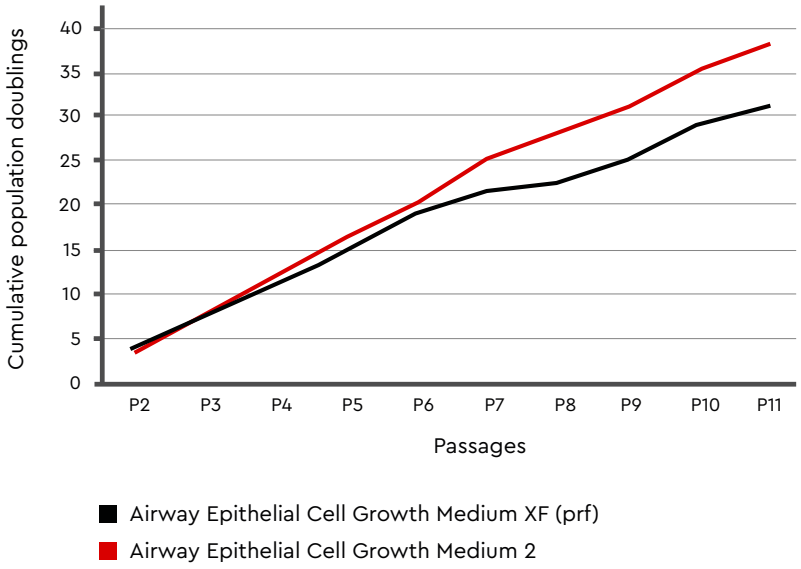

Fig. 6: Long-term expansion of HTEpC in our airway culture medium. *Primary HTEpC were isolated from healthy human tissue and seeded in Airway Epithelial Cell Growth Medium 2 or Airway Epithelial Growth Medium XF (prf). Cells were expanded and cryopreserved. Thawed cells were seeded at a density of 5,000 cells per cm² and then serially passaged until they reached senescence. Representative data showing HTEpC growth from thawing (passage 2) until senescence (passage 11).*

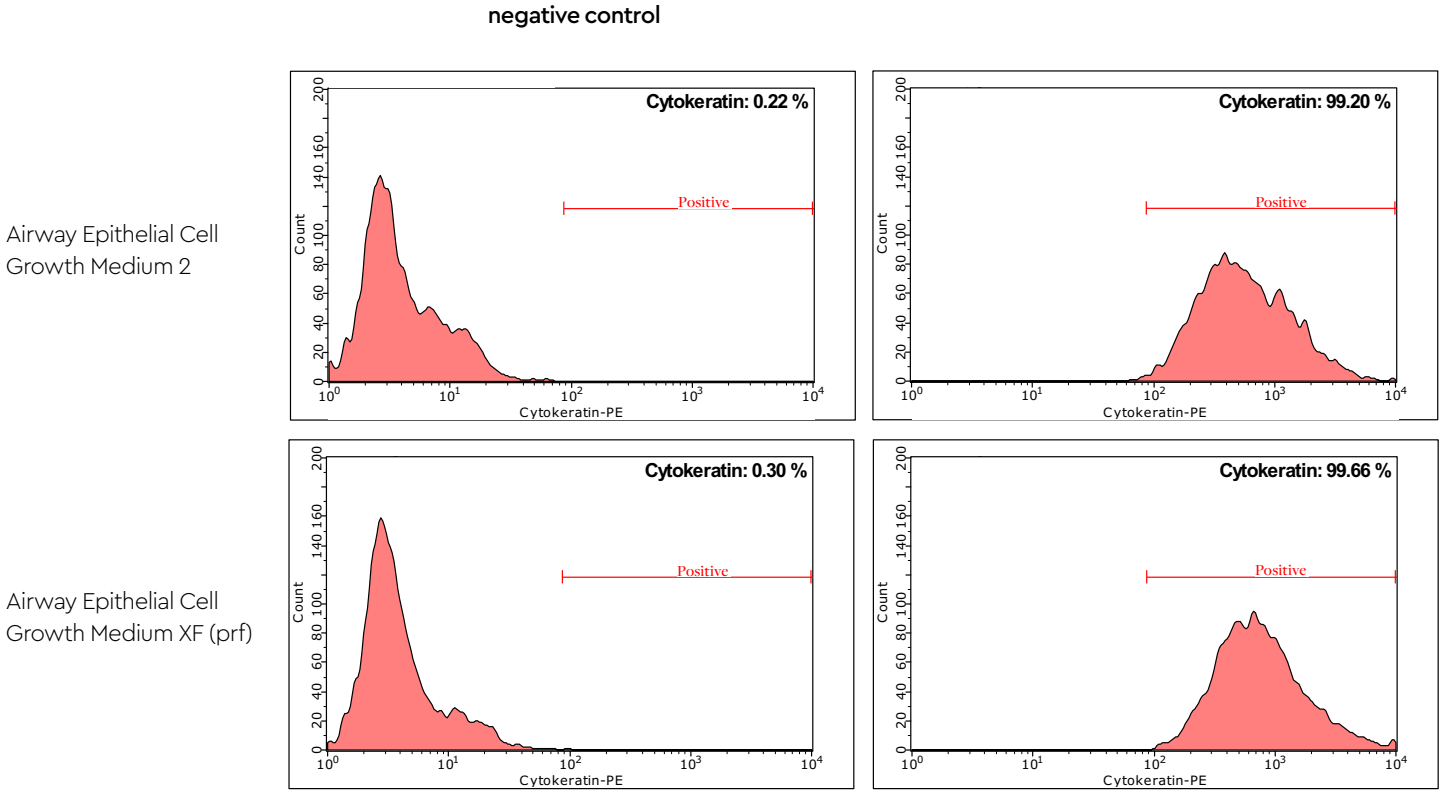

Fig. 7: Cell identity of isolated HTEpC in our airway culture medium. *Primary HTEpC were isolated from healthy human tissue and seeded in Airway Epithelial Cell Growth Medium 2 or Airway Epithelial Growth Medium XF (prf). Cell identity was analyzed in passage 3 using FACS after cytokeratin antibody staining.* ***Left side:*** *Negative control plots show the FACS gating strategy of unstained cells.* ***Right side:*** *Cells stained with cytokeratin antibody. More than 90% of the cells are cytokeratin-positive, indicating a pure epithelial cell expansion without unwanted cell contamination.*

Trademark references

Material

Product	Size	Catalog number
Airway Epithelial Cell Growth Medium 2	500 ml	C-21040
Airway Epithelial Cell Growth Medium XF (prf)	500 ml	C-21050
Human Bronchial Epithelial Cells (HBEpC)	500,000 cryopreserved cells	C-12640
	500,000 proliferating cells	C-12641
Human Tracheal Epithelial Cells (HTEpC)	500,000 cryopreserved cells	C-12644
	500,000 proliferating cells	C-12645
Human Nasal Epithelial Cells (HNEpC)	500,000 cryopreserved cells	C-12620
	500,000 proliferating cells	C-12621
HEPES Buffered Saline Solution	250 ml	C-40020
Dulbecco's PBS, without Ca^{2+}/Mg^{2+}	500 ml	C-40232
DetachKit	125 ml	C-41210

Additional products

Product	Size	Catalog number
BioCoat® Collagen I Cellware 150 mm Dish with Lid (Corning®)	_	354551
Nunclon™ Delta Surface Nunc Dish 150 × 20 (Thermo Scientific)	_	168381
Falcon® Easy Grip TC-treated (Corning®)	_	353004

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