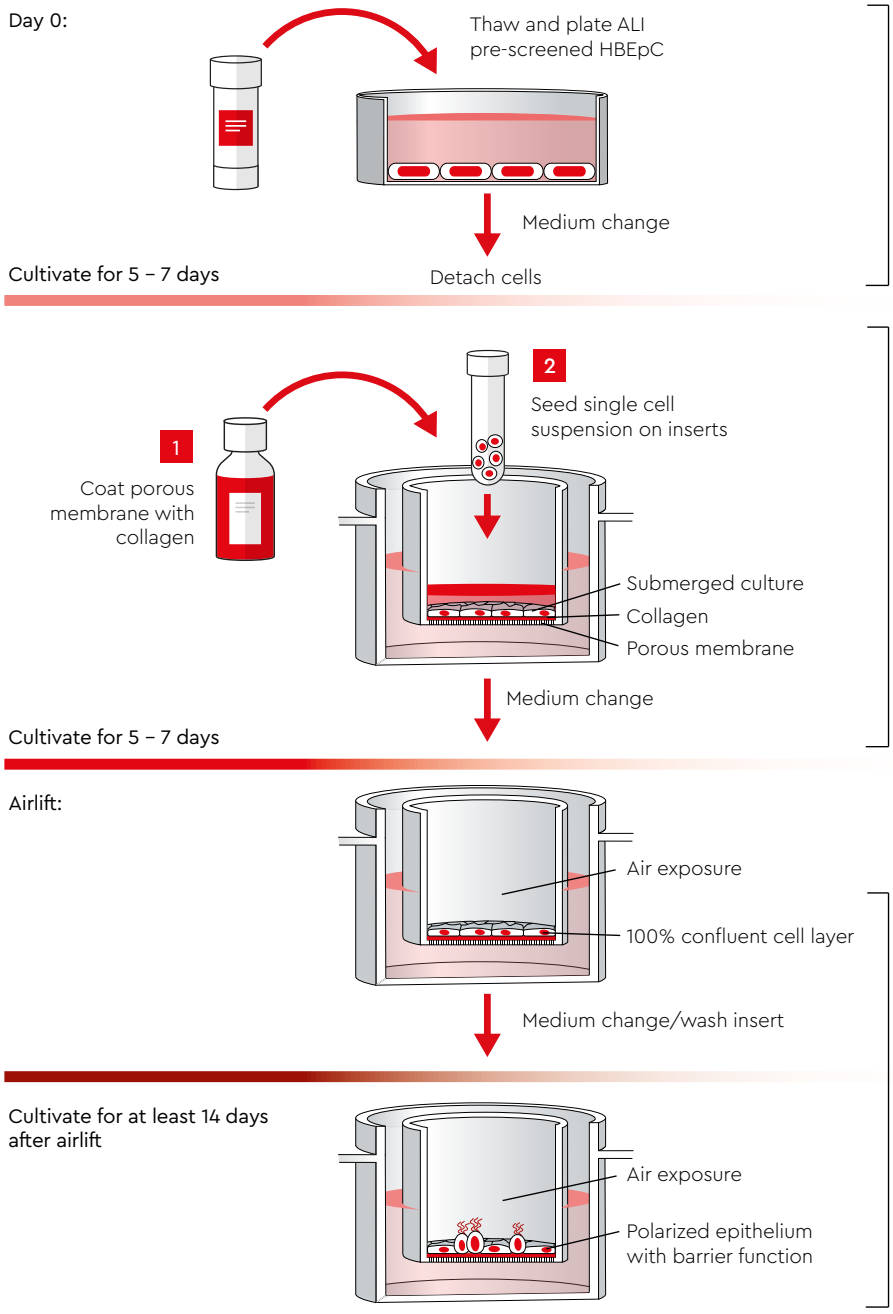
Air-Liquid Interface Culture System for standardized respiratory research: A serum- and BPE-free system

Application note

Generation of a stable and functional 3D human airway model with primary human bronchial epithelial cells using our Air-Liquid Interface Culture System.



2D phase:
Expansion in Airway Epithelial Cell Growth Medium 2

See part A of protocol

3D phase (submerged):
Proliferation in Airway Epithelial Cell Growth Medium 2 on collagen type I coated permeable cell culture inserts

See part B of protocol

3D phase (airlift):
Differentiation in ALI-Airway medium on permeable cell culture inserts with air exposure

See part C of protocol

Fig. 1: Schematic overview of culture phases for differentiation of HBEpC in the Air-Liquid Interface (ALI-Airway) culture system. *The workflow can be divided into three phases:* ***A)*** *Expansion of human primary cells in 2D culture on plastic.* ***B)*** *Reseeding and expansion of the cells in 3D culture on collagen type I-coated porous membranes in submerged culture.* ***C)*** *Induction of the differentiation phase by airlift in ALI-Airway medium. The formation of a tight epithelial barrier occurs during the differentiation phase, which is maintained for at least 14 days after airlift.*

Background

Airway epithelial models are a central tool in respiratory research, including drug testing, toxicology, studies, and disease modeling for conditions such as asthma, COPD, and cystic fibrosis.[1–3] However, traditional 2D culture systems fail to recapitulate the architecture and function of the human respiratory epithelium.[4]

Air-liquid interface (ALI) culture systems bridge this gap by enabling the development of differentiated pseudostratified airway epithelium that mirrors *in vivo* physiology. When cultured at the air-liquid interface, primary human bronchial epithelial cells undergo mucociliary differentiation, forming an epithelial layer that contains basal cells, mucus-producing goblet cells, and motile ciliated cells.[5–7] This differentiation is supported by epithelial polarization and the formation of apico-lateral junctional complexes, including tight and adherens junctions, which are critical for barrier integrity.[8,9]

Barrier function in ALI cultures can be quantitatively assessed using transepithelial electrical resistance (TEER), a non-invasive method that measures ohmic resistance or impedance across the epithelial layer.[9] TEER values are widely used as indicators of epithelial integrity and can be monitored in real time without compromising cell viability.

Traditional ALI systems often rely on serum-containing media, which introduce variability due to undefined components and batch-to-batch differences.[10] Our serum- and bovine pituitary extract (BPE)-free ALI culture system addresses these challenges by providing a chemically defined environment that ensures reproducibility and supports regulatory compliance.

The system includes a three-phase workflow (see Figure 1):

- **2D expansion phase** using Airway Epithelial Cell Growth Medium 2 (C-21040), where HBEpC are expanded.
- **3D submerged phase** on collagen type I-coated porous membrane inserts, where cells proliferate under submerged conditions until confluence is reached.
- **3D differentiation phase (airlift)** using the Air-Liquid Interface Medium (ALI-Airway, C-21080), where cells are exposed to air on the apical side and undergo differentiation.

When cultured under these conditions, HBEpC form a high-integrity epithelial barrier with TEER values typically exceeding 500 Ω·cm² and maintained for at least 14 days, making the system suitable for extended experimental protocols. This model supports a wide range of downstream applications, including permeability studies, host-pathogen interaction assays, cytokine release profiling, and compound screening.

Protocol for complete serum- and BPE-free ALI culture system - Part A

2D expansion of ALI pre-screened Human Bronchial Epithelial Cells

This section outlines the procedure for thawing and expanding ALI pre-screened HBEpC in 2D monolayer culture on plastic surfaces.

I. Materials

- ALI pre-screened Human Bronchial Epithelial Cells (ALI pre-screened HBEpC; C-12640)
- Airway Epithelial Cell Growth Medium 2 (C-21040)
- Phosphate Buffered Saline w/o Ca^{++}/Mg^{++} (PBS, C-40232)
- Cell culture vessel (e.g., Falcon® or Corning®)
- DetachKit (C-41210; 125 ml) containing the following products: HEPES Buffered Saline Solution (BSS), 0.04% Trypsin/0.03% EDTA, and Trypsin Neutralization Solution (0.05% Trypsin Inhibitor in 0.1% BSA)

II. Protocol

To prepare the Airway Epithelial Cell Growth Medium 2, thaw the SupplementMix at 15–25°C. Under aseptic conditions, mix the supplement solution by carefully pipetting up and down, then transfer the entire content to the 500 ml bottle of growth medium. Close the bottle and swirl gently until a homogeneous mixture is formed. The complete medium should be protected from light and has a shelf life of 6 weeks when stored at 4–8°C. Before use, calculate the amount of medium needed for starting the culture and prewarm only the required aliquot to room temperature, while keeping the remaining medium refrigerated.

1

Thaw the ALI pre-screened HBEpC

Remove the cryovial from liquid nitrogen and transport it on dry ice. Under a laminar flow bench, briefly loosen the cap to release pressure, then retighten. Thaw the vial in a 37°C water bath for 2 minutes. Rinse the outside of the vial with 70% ethanol before placing it under the laminar flow bench. Aspirate any residual ethanol from the threads, open the vial carefully, and transfer the cell suspension into a centrifuge tube containing 9 ml of Airway Epithelial Cell Growth Medium 2.

Note: Our cryopreserved cells are frozen in Cryo-SFM Plus (C-29920), which contains DMSO. Work quickly to minimize exposure of the cell suspension to DMSO, as cells are highly sensitive immediately after thawing.

2

Collect the cells and determine cell number and viability

Gently mix the 10 ml cell suspension and take an aliquot for cell counting. Centrifuge the 10 ml cell suspension (300 x g for 3 minutes) and aspirate the supernatant. Resuspend pellet in Airway Epithelial Cell Growth Medium 2. Count the cells using your standard method.

3

Seed the cells

Use a seeding density of 5,000 cells per cm^2 in growth medium (180 µl per cm^2). Swirl the culture vessel gently to distribute the cells evenly, then place it in an incubator (37°C, 5% CO_2).

4

Maintain the culture

After 16–24 hours, examine the cultures under the microscope to confirm cell adherence. Replace the growth medium every Monday, Wednesday, and Friday. Perform the first medium change no earlier than 24 hours after seeding.

Note: Avoid confluence >90% for HBEpC. The cells can become contact-inhibited, resulting in slower proliferation after passaging.

5

Subcultivate the expanded cells

When cultures reach 70–90% confluence, aspirate the medium carefully and wash the cells with 0.15 ml HEPES BSS per cm^2 of vessel surface. Gently agitate the vessel for 15 seconds, then aspirate. Optionally, Dulbecco's PBS w/o Ca^{++}/Mg^{++} may be used instead. Cover the cells with 50 µl Trypsin/EDTA Solution per cm^2 and incubate at 37°C for 4 minutes.

6

Harvest the detached cells

Monitor the cell round-up process under a microscope. Tap the side of the culture vessel to enhance the cell dissolution from the plastic. When cells are detached, add 50 µl Trypsin Neutralization Solution per cm^2 and gently agitate. Resuspend the cells and transfer the cell suspension into a centrifuge tube containing an appropriate volume of Airway Epithelial Cell Growth Medium 2. Rinse the culture vessel with HEPES BSS (optional Dulbecco's PBS w/o Ca^{++}/Mg^{++}) and pool the cell suspension into the centrifuge tube. Examine the culture vessel under a microscope to confirm that all the airway epithelial cells have been harvested. Centrifuge the cell suspension at 300 x g for 3 min.

7

Collect the cells and determine the cell number and viability

Carefully discard the supernatant and resuspend the pellet by gently pipetting up and down in an appropriate volume of Airway Epithelial Cell Growth Medium 2. Remove an aliquot of the resuspended cells to determine cell number and viability using your standard method.

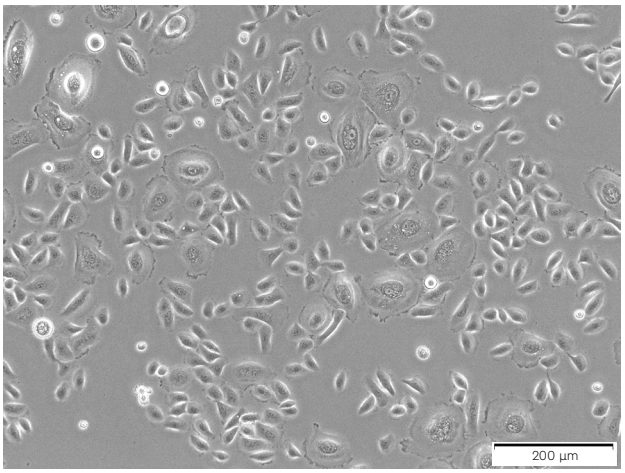


Fig. 2: Typical morphology of HBEpC in 2D culture. *The cells were expanded in PromoCell Airway Epithelial Cell Growth Medium 2 until they reached 70–90% confluence. The image was taken 5 days after thawing, under phase contrast microscopy at 10x magnification (scale bar = 200 µm).*

Protocol for complete serum- and BPE-free ALI culture system - Part B

Subcultivation and re-plating of HBEpC on precoated semipermeable cell culture inserts

This section outlines the procedure for seeding HBEpC at high density on collagen type I-coated Transwell® inserts and maintaining them as submerged 3D cultures until confluence is reached.

I. Materials

- Airway Epithelial Cell Growth Medium 2 (C-21040)
- Phosphate Buffered Saline w/o Ca^{++}/Mg^{++} (PBS, C-40232)
- Collagen Type I Solution (Rat Tail) (we strongly recommend using product with catalog number 354236 from Corning® Inc.)
- 6.5 mm Transwell® inserts, 0.4 µm pore size, tissue culture treated polyester membrane polystyrene plates (we strongly recommend Costar®, product number 3470-Clear from Corning® Inc.)

II. Protocol

1

Coat the Transwell® porous membrane inserts with collagen type I solution

On the day of use, prepare a collagen type I working solution at 30 µg/ml in PBS w/o Ca^{++}/Mg^{++}. Since collagen is viscous, prewarm the solution to facilitate easier pipetting. Calculate the required amount of collagen working solution. If you plan to perform TEER measurements, prepare additional inserts as blank controls by coating them without seeding cells. For 6.5 mm inserts (growth area = 0.33 cm^2), add 100 µl of the solution to the apical chamber. Distribute the liquid evenly by gently rocking the plate from side to side and front to back; avoid circular swirling, which can cause uneven coating. To prevent evaporation, you can fill the outer walls of the plate with PBS or other sterile liquid. Incubate the coated plate for 45 minutes at 37°C in a humidified incubator with 5% CO_2.

2

Wash the coated inserts before seeding and prewarm the plate with medium

After the inserts have been collagen-coated, aspirate the coating solution and wash each insert once with 150 µl of prewarmed PBS w/o Ca^{++}/Mg^{++}. Do not allow the coated inserts to dry out. Add 500 µl of growth medium into the basal chamber and 100 µl of growth medium into the apical chamber, and prewarm the plate in the incubator (37°C, 5% CO_2).

Note: If inserts are not used the same day, they may be stored at 4–8°C for up to four weeks, provided they remain hydrated with PBS or collagen solution. Do not allow membranes to dry at any point, as this will compromise performance.

3

Prepare the cell suspension

Approximately 5–7 days after thawing, the HBEpC should reach 70–90% confluence. Detach and collect the cells according to the subcultivation procedure described in Part A and prepare a single-cell suspension in Airway Epithelial Cell Growth Medium 2. Determine cell number and viability using your standard method.
Calculate the number of cells required to achieve a seeding density of 150,000 living cells/cm^2. For 6.5 mm Transwell® inserts with a surface area of 0.33 cm^2, this corresponds to 49.500 cells per insert. Adjust the suspension to a final concentration of 500,000 cells/ml in Airway Epithelial Cell Growth Medium 2.

4

Seed the cells on the inserts

Remove the precoated Transwell® plate from the incubator and place it under the laminar flow bench. Aspirate the 100 µl medium from the apical chamber of each insert, and keep the 500 µl medium in the basal chamber unchanged. Carefully pipette 100 µl of the cell suspension (500,000 cells/ml) into the apical chamber of each insert. For optimal cell distribution, gently rock the plate side to side and front to back immediately after seeding, but avoid making swirling motions.

Note: When planning to perform TEER measurements, prepare at least one blank insert by adding medium to both chambers without seeding cells. This blank serves as a background resistance control.

5

Expand the cells in submerged culture

Check the cultures under a microscope 24 hours after seeding to assess attachment and confluence. By this point, cells typically reach 80–100% confluence. Replace the medium 24 hours after seeding in both chambers by gently tilting the plate and carefully pipetting to avoid disturbing the cell layer. Refill the basal chamber with 500 µl and the apical chamber with 100 µl of prewarmed Airway Epithelial Cell Growth Medium 2 (see Figure 3A). Continue changing the medium in both chambers every Monday, Wednesday, and Friday. Change the medium in the morning on Mondays, and in the evening on Fridays. Monitor the cultures to confirm continued confluence and uniform coverage of the membrane. Maintain the cells under submerged conditions for at least five days, even if complete confluence is achieved earlier. This extended culture period is important to allow the epithelial layer to close completely and form stable cell-cell contacts before initiating the airlift differentiation phase.

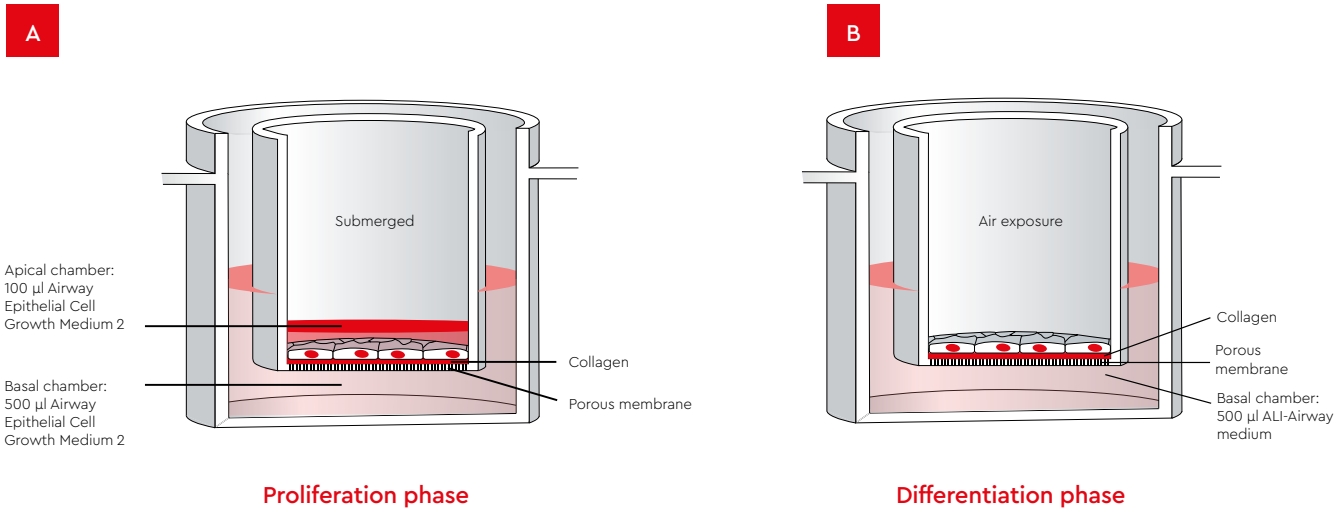


Fig. 3: Schematic overview of HBEpC culture on collagen-coated permeable inserts. A) Submerged phase: *Cells are grown on collagen I-coated porous membranes with Airway Epithelial Cell Growth Medium 2 in both the apical and basal chambers, allowing for attachment and expansion.* ***B) Airlift phase:*** *Upon transition to 3D airlift culture, the apical medium is removed, and the cell layer is exposed to air, while the basal medium is replaced with ALI-Airway medium to initiate differentiation.*

Protocol for complete serum- and BPE-free ALI culture system - Part C

Differentiation of HBEpC at the air-liquid interface

This section describes the 3D culture of HBEpC on permeable cell culture inserts in ALI-Airway to promote differentiation over 2–4 weeks under air-exposed conditions.

I. Materials

- Air-Liquid Interface Medium (ALI-Airway, C-21080)
- Gentamicin-Sulfate solution with a final concentration of 50 µg/ml in the medium
- Phosphate Buffered Saline w/o Ca^{++}/Mg^{++} (PBS, C-40232)

Note: Use aseptic techniques and a laminar flow bench.

II. Preparation of Air-Liquid Interface Medium

Our Air-Liquid Interface Medium (ALI-Airway, C-21080) is designed to support the barrier-forming function of ALI pre-screened HBEpC on permeable cell culture inserts under airlift conditions. The medium does not contain adherence factors, so it must be used with collagen-coated inserts. To prepare the medium, thaw the SupplementMix at 15–25°C. Aseptically mix the supplement by carefully pipetting up and down. Then, transfer the entire content of the SupplementMix into the 500 ml bottle of basal medium. Close the bottle and swirl gently until a homogenous mixture is formed. We recommend adding 50 µg/ml of gentamicin for long-term cultivation, especially if you want to perform TEER measurements using an electrode pair. After adding the SupplementMix, the shelf life of the ALI-Airway medium is 6 weeks. Store the complete growth medium at 4–8°C and protect it from light. At the time of use, allow the medium to warm up to room temperature (do not pre-warm at 37°C). ALI-Airway contains light-sensitive components, and we recommend protecting it from light.

III. Protocol

1

Transition cultures to the airlift phase

Initiate the air-liquid interface culture once the cell layer has reached complete confluence on permeable inserts (typically 5 days after seeding). If full confluence is not observed, continue submerged culture for an additional day and recheck before proceeding. To initiate the airlift, carefully aspirate the Airway Epithelial Cell Growth Medium 2 from both the upper and lower chambers. Add 500 µl of ALI-Airway to the basal chamber only; leave the apical chamber empty to expose the cells to air, which stimulates differentiation (see Figure 3B).

Note: Some cells may form 3D nodules during this stage (Figure 4); this is normal, and the nodules will detach during subsequent medium changes.

2

Maintain cultures under airlift conditions

Change the ALI-Airway in the basal chamber every 2–3 days, keeping the apical chamber air-exposed throughout.

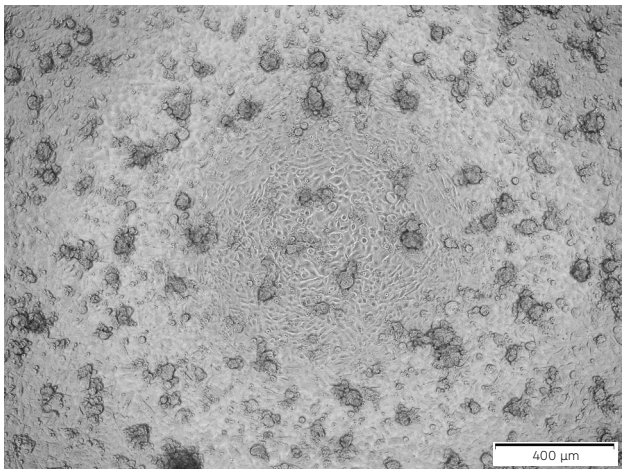


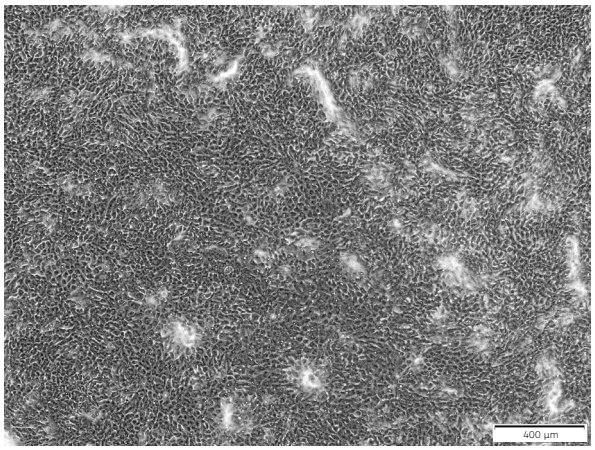
Fig. 4: Growth and morphology of HBEpC in 3D culture in serum- and BPE-free Airway Epithelial Cell Growth Medium 2. *Microscopy images of HBEpC in 3D culture before airlift. Cells were grown for five days in Airway Epithelial Cell Growth Medium 2 as a submerged culture on collagen I-coated Transwell® inserts (Corning®, Inc.). The picture was taken at the time point of medium switch to ALI-Airway under airlift condition (4x magnification, scale bar = 400 µm).*

3

Differentiation until week 4

When using ALI pre-screened HBEpC and following the recommended protocol, TEER values ≥ 500 Ω·cm² can be maintained for at least two weeks. Differentiation is typically completed on day 28 after airlifting. In this serum- and BPE-free ALI culture system, cells form a confluent epithelial layer with high structural integrity and sustained viability, providing a physiologically relevant model of the human airway epithelium (Figure 5).

A



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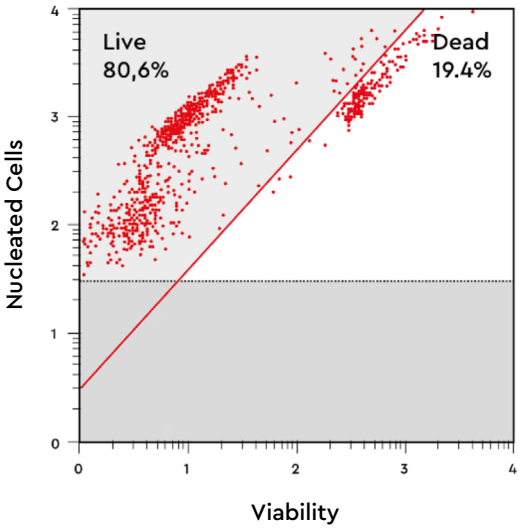

Fig. 5: Integrity and viability of HBEpC in a complete serum- and BPE-free ALI culture system. *After 2D expansion and reseeding on collagen-coated inserts, HBEpC differentiated in ALI-Airway form a confluent, viable epithelial layer.* ***A)*** *Microscopy image of a confluent cell layer after 28 days under airlift conditions showing typical morphology (4x magnification, scale bar = 400 µm).* ***B)*** *Representative cell viability analysis determined using a Muse® Cell Analyzer (Cytek® Biosciences). Viability should be greater than 70%.*

Protocol for complete serum- and BPE-free ALI culture system - Part D

Assessment of epithelial barrier integrity by TEER measurement

This section describes the procedure for monitoring the formation and maintenance of tight epithelial barriers using TEER measurements with a voltohmmeter.

I. Materials

- EVOM® voltohmmeter with electrode (World Precision Instruments®)
- 1.000 Ω test resistor (World Precision Instruments®)
- 70% EtOH
- Phosphate Buffered Saline w/o Ca^{++}/Mg^{++} (PBS, C-40232)
- Air-Liquid Interface Medium (ALI-Airway, C-21080)

II. Protocol

1

Prepare for TEER measurement

Carefully aspirate the medium in both chambers. Add 800 µl of prewarmed ALI-Airway to the basal chamber and 250 µl to the apical chamber. Incubate the plate in an incubator (37°C, 5% CO_2) for 30 minutes to allow for temperature equilibration before measurement.

2

Perform TEER measurements with EVOM™ voltohmmeter

Place the EVOM™ voltohmmeter under the laminar flow bench. Disinfect the electrode pair with 70% ethanol, then rinse by sequentially immersing the electrodes in three wells of an empty 24-well plate, each containing 1.7 ml of prewarmed PBS w/o Ca^{++}/Mg^{++}. Calibrate the instrument according to the supplier's instructions.
Establish a baseline by measuring resistance in a "blank" insert without cells, ensuring the shorter electrode is positioned in the upper chamber (Figure 6). Typical blank resistance values range from 150 to 350 Ω and should not exceed 400 Ω. Proceed to measuring the electrical resistance of the culture samples. Perform measurements promptly to avoid temperature-related fluctuations. Rinsing the electrodes between samples is generally not required, unless recommended by the manufacturer for specific applications.

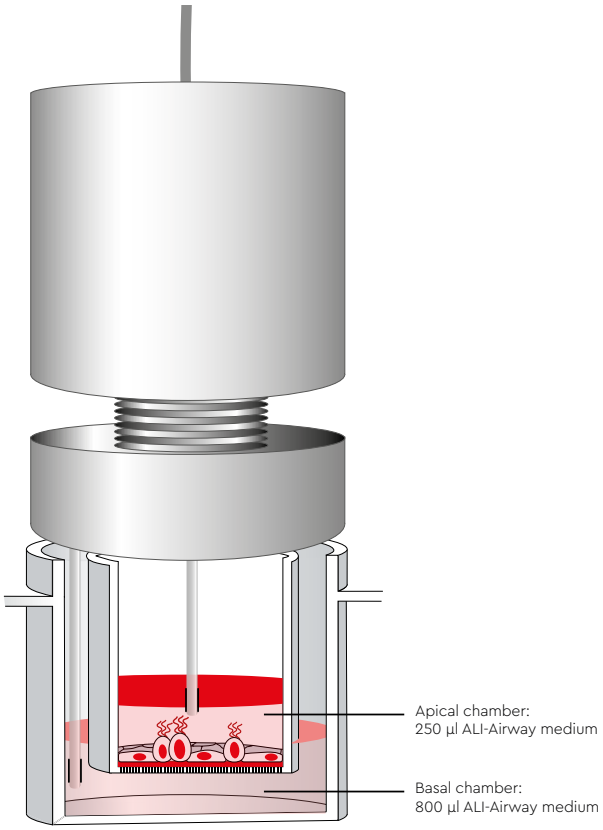

Fig. 6: TEER measurements using EVOM™. *Measurements using the EVOM™ voltohmmeter should be conducted under a laminar flow bench. Once EVOM™ is calibrated following the supplier's instructions, immerse the electrode pair in each well to perform the TEER measurements.*

3

Return to airlift condition and cultivate until endpoint

After TEER measurement, carefully aspirate 250 µl of ALI-Airway from the upper chamber and 800 µl from the lower chamber. Replenish the basal chamber with 500 µl of prewarmed ALI-Airway and leave the upper chamber air-exposed. Continue replacing the medium in the lower chamber with 500 µl of medium every 2–3 days until the final endpoint measurement.

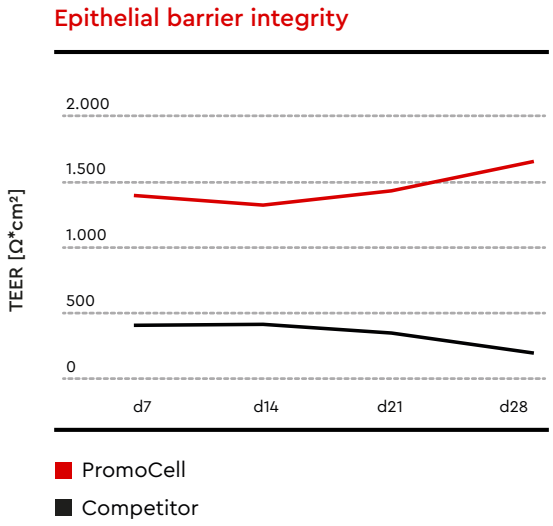

Fig. 7: TEER values of HBEpC in our serum- and BPE-free ALI-Airway compared with competitor medium. *HBEpC were thawed and seeded in serum-free and BPE-free Airway Epithelial Cell Growth Medium 2 or airway growth medium of a competitor. Cells were detached and reseeded on collagen I-coated 24-well Transwell® plates (3470-clear, Corning®) in respective growth medium. After five days, the medium was switched to our serum- and BPE-free ALI-Airway or competitor ALI medium for 28 days. Epithelial barrier function was measured once a week using a voltohmmeter (EVOM™ World Precision Instruments). The graph shows representative TEER data over 4 weeks of HBEpC culture in two different media. Our serum- and BPE-free ALI-Airway consistently outperformed the competitor throughout the entire differentiation period.*

Troubleshooting

Problem	Possible causes	Solutions
Poor cell attachment	Inadequate collagen coating or use of cell culture plastic from a supplier other than the recommended one (we recommend Corning®)	Ensure 45-minute coating time; verify collagen concentration. Use Transwell® plastic from Corning®.
Low TEER values	Cells were not fully confluent before airlift	Extend the submerged culture phase until complete confluence. If confluence is reached, allow a few more days in submerged culture to promote stronger cell-cell adhesion. Cells will not proliferate further in ALI-Airway medium, since it lacks growth factors.

Problem	Possible causes	Solutions
Cell detachment	Mechanical disruption or unstable culture conditions	Handle inserts carefully and avoid pipette contact with the cell layer. Check incubator conditions, including humidity, temperature, and CO_2 stability, as these parameters can influence the pH buffering system of the cell culture medium. In addition, accumulation of cytotoxic products (e.g., ammonia) may impact cell behavior and should be considered.
Contamination	Inadequate sterile technique	Review aseptic handling procedures and include gentamicin in the medium if required.

Best practices

- **Use the specified insert format:** Employ the recommended Transwell® insert type (Costar® #3470-Clear) to maintain consistency across experiments.
- **Follow a defined medium change schedule:** Replace culture medium at regular intervals (every 2–3 days) to maintain cell health and stability.
- **Monitor cell morphology regularly:** Routine microscopic checks help to detect early signs of culture stress and morphological changes.
- **Standardize TEER measurement timing:** Perform transepithelial electrical resistance (TEER) assessments at consistent, predefined time points to enable reliable comparisons.
- **Maintain comprehensive documentation:** Record culture conditions, procedural deviations, and performance metrics in detail to facilitate traceability and reproducibility.

Trademark references

Material

Product	Size	Catalog number
Airway Epithelial Cell Growth Medium 2	500 ml	C-21040
Air-Liquid Interface Medium (ALI-Airway)	500 ml	C-21080
Human Bronchial Epithelial Cells (HBEpC), ALI pre-screened*	500,000 cryopreserved cells	C-12640
HEPES Buffered Saline Solution	250 ml	C-40020
Dulbecco's PBS, w/o Ca^{2+}/Mg^{2+}	500 ml	C-40232
DetachKit	125 ml	C-41210

***Note:** Our HBEpC are now also available from Air-Liquid Interface pre-screened, HLA-typed, and COPD/Asthma donors.*

Related products

Product	Size	Catalog number
Collagen Type I (Rat Tail) (Corning®)	3–4 mg/ml	354236
EVOM® (World Precision Instruments®)	–	–
Falcon® Easy Grip TC-treated (Corning®)	–	353004

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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

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