# Air-Liquid Interface Culture System for Standardized Respiratory Research

## Application Note

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

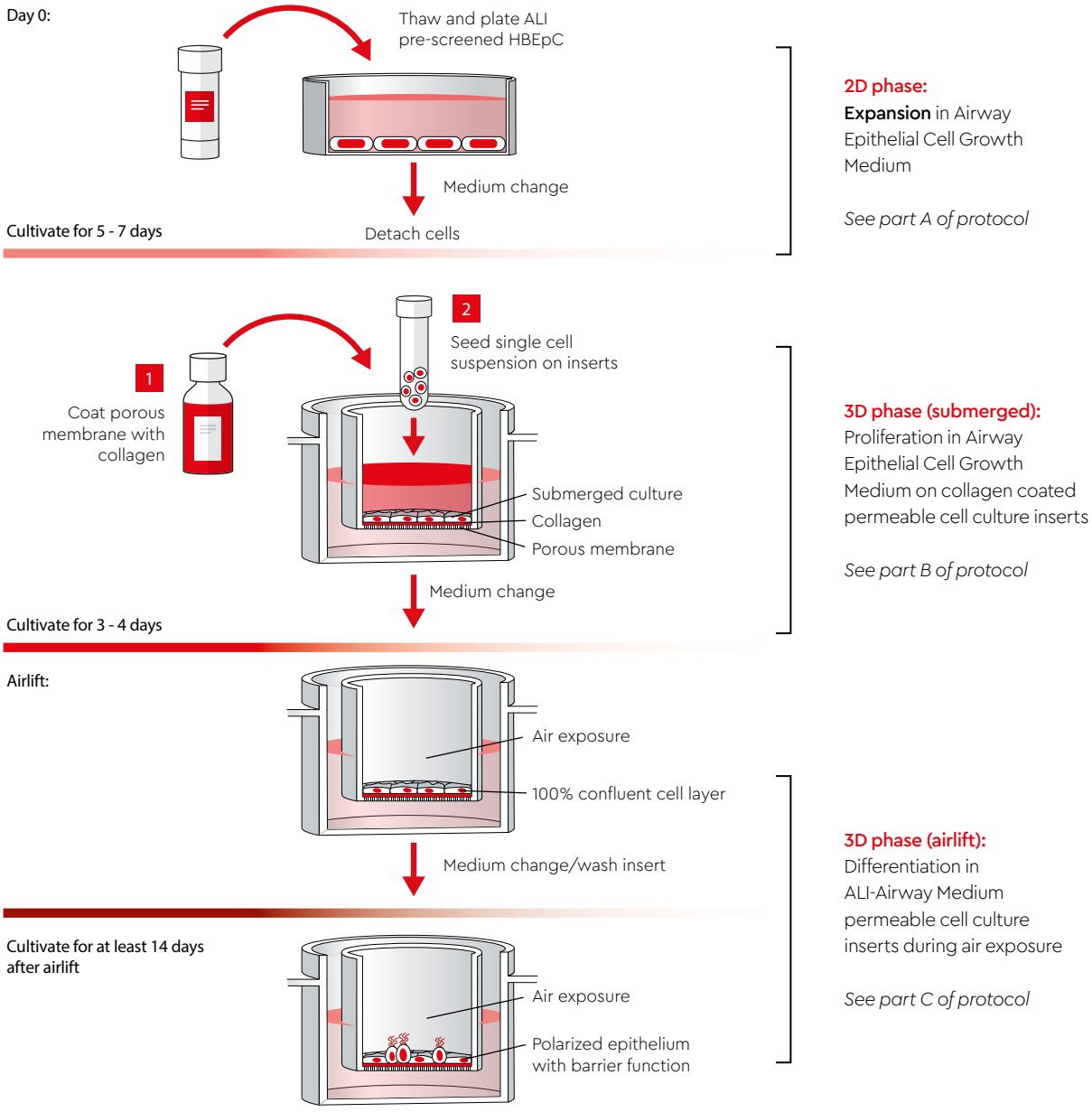



***Fig. 1: Schematic overview of culture phases for differentiation of HBEpC in the PromoCell ALI culture media system.*** *The workflow can be divided into various phases:* ***A,*** *Expansion of human primary cells in 2D culture on plastic.* ***B,*** *Re-seeding and expansion of the cells in 3D culture 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 in the differentiation phase lasting at least 14 days post-airlift. For the detailed steps, please see the protocol below post-airlift. For the detailed steps, see the following protocol.*

## Background

The transcriptional profile of differentiated primary cells grown at the air-liquid interface (ALI) very closely resembles the in vivo airway epithelium, suggesting that the use of primary cultures and the presence of an air-liquid interface are important to recapitulate airway epithelial biology. In addition, the close similarity between cells of tracheal and bronchial origin within and between different human donors suggests a robust expression profile that is specific to airway cells [1].

In vitro ALI models have therefore been recommended for studying the physiological and pathophysiological responses of the respiratory tract, molecular events, and modes of action and interaction of different cell types [2]. Well-differentiated in vitro airway epithelial cultures are characterized by the formation of a pseudostratified epithelium and a barrier function between adjacent environments. Although airway epithelial cells do not differentiate in 2D culture on plastic, they can undergo mucociliary differentiation when grown on porous membranes at an air-liquid interface. The air-liquid interface permits polarization of epithelial cells by supporting their differentiation [3].

An epithelial barrier is generated by a high-integrity apico-lateral junctional complex composed of tight and adherens junctions. The integrity of these tight junction dynamics in cell culture models of epithelial monolayers can be quantified by measuring the transepithelial electrical resistance (TEER). TEER values are widely accepted as strong indicators of the integrity of the cellular barriers. TEER measurements can be performed in real time without cell damage and are generally based on measuring ohmic resistance or impedance [4], [5], [6], [7], [8].

ALI cultures can also be used for multiple readouts, e.g. viability assays, analysis of virus infection and respiratory disease mechanisms, cytokine release, and expression of messenger RNA.

We have developed a standardized Air-Liquid Interface Culture System that ensures epithelial barriers of highly viable primary human bronchial epithelial cells (HBEpC) over a period of at least 14 days when cultured in 3D on a porous membrane. The barrier function can be quantified by measuring TEER (> 500 Ω*cm$^2$ for at least 14 days in culture).

The PromoCell Air-Liquid Interface Culture System workflow is divided into a 2D expansion phase and a 3D differentiation phase (see Figure 1). After 2D expansion of ALI pre-screened HBEpCs in Airway Epithelial Cell Growth Medium (C-21060), the cells have to be seeded on permeable cell culture inserts as submerged cultures and allowed to grow until they reach confluence. Quality control evaluated ALI pre-screened HBEpC lots are tested for proper barrier function.

Differentiation of ALI pre-screened HBEpC is stimulated by changing to the Air-Liquid Interface Medium (ALI-Airway, C-21080) and exposure to air. The ALI-Airway medium lacks attachment factors, and collagen type I is therefore a prerequisite for optimal cell attachment. The ALI-Airway medium consists of a Basal Medium and a BPE- and serum-free SupplementMix that enhances the barrier-forming function of HBEpCs (see Figure 1). Our ALI pre-screened HBEpC are available on request by contacting our Scientific Support.

The following application note describes the ALI cell culture procedure in detail and recommended TEER measurement technology.

# Air-Liquid Interface Culture System Protocol Part A

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

The protocol in section A describes the procedure for thawing and expanding of the required amount of ALI pre-screened HBEpC in 2D culture on plastic.

## I. Materials

- ALI pre-screened Human Bronchial Epithelial Cells (HBEpC) C-12640
- Airway Epithelial Cell Growth Medium, containing the Basal Medium and either SupplementMix (Ready-to-use; C-21060) or SupplementPack (Kit; C-21160).
- Cell culture vessel (e.g. Falcon®; Corning® Inc.)
- 6.5 mm of Transwell® inserts, 0.4 µm pore size, tissue culture treated polyester membrane polystyrene plates (we strongly recommend Costar® from Corning® Inc., product number 3470-Clear), alternative products see Material List on page 10)
- Water bath at 37°C
- Timer
- Cell counting equipment

*Use aseptic techniques and a laminar flow bench.*

# II. Protocol

To prepare the medium, thaw the SupplementMix or SupplementPack at 15 – 25°C. Aseptically mix the supplement by carefully pipetting it up and down. Transfer all components to the 500 ml bottle of Basal Medium. Close the bottle and swirl gently until a homogenous mixture is formed. After addition of the supplement(s) to the Basal Medium, its shelf life (complete growth medium) is 6 weeks. Store the complete growth medium at 2 – 8°C.

1

## Thaw the ALI pre-screened HBEpC

Remove the cryovial from liquid nitrogen and transport the vial on dry ice. Under a laminar flow bench, release the pressure of the vial by briefly twisting the cap counterclockwise by a quarter turn and then retightening it. Allow the cell suspension to thaw in a water bath at 37°C for 2 minutes. Rinse the vial with 70% EtOH and place it under a laminar flow bench. Aspirate the ethanol from the threads of the screw cap. Carefully open the cryovial. Transfer the cell suspension to the cell culture vessels containing the prewarmed medium from step 1.

**Note:** Our cryopreserved cells are frozen in Cryo-SFM C-29910, which contains DMSO. Work quickly to prevent a longer incubation of the cell suspension in Cryo-SFM, because the cells are very sensitive after thawing.

2

## Incubate the cells

Gently swirl the vessel containing the cell suspension and place it in an incubator (37°C, 5% $CO_2$). After 16 – 24 hours, check the cell adherence under a microscop and replace the growth medium. There should be only a few floating cells.

3

## Cultivate cells while regularly changing the medium

Change the medium every two to three days (e.g. Mon-Wed-Fri). Use prewarmed complete Airway Epithelial Cell Growth Medium (180 µl medium/$cm^2$). Regularly check the confluence of the cells. Once they have reached 70 – 80% confluence, passage the cells.

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

4

## Subcultivate the expanded cells

Once the cells have reached 70 – 80% confluence, subculivate the cells as described in section B. The required confluence can typically reached 5 – 7 days after thawing. The morphology of the cells should be the typical cobblestone pattern of epithelial cells (see Figure 2).

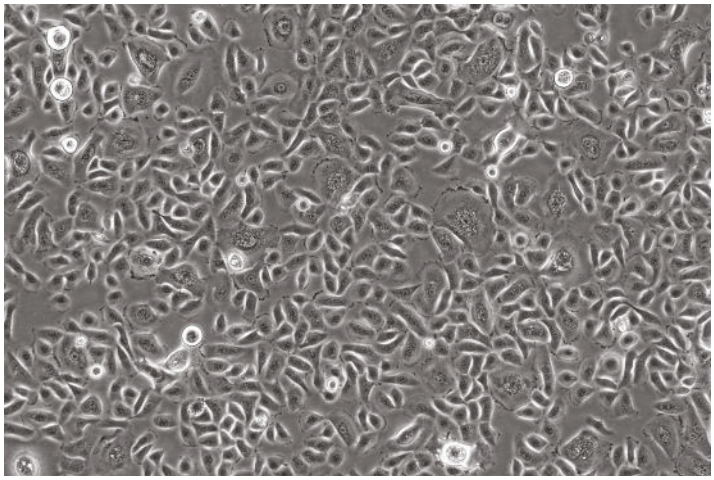


***Fig. 2: Typical morphology of HBEpC in 2D culture.*** *The cells were expanded in PromoCell Airway Epithelial Cell Growth Medium until they reached 70 – 80% confluence. The image was taken 7 days after seeding at 10x magnification.*

# Air-Liquid Interface Culture System Protocol Part B

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

This section describes detachment from 2D culture and seeding of HBEpC at high density on collagen type I precoated Transwell® inserts as a submerged 3D culture.

## I. Materials

- Phosphate Buffered Saline w/o $Ca^{++}$/$Mg^{++}$ (PBS, C-40232)
- 0.04 % Trypsin/0.03% EDTA (Trypsin/EDTA, C-41010)
- 0.05 % Trypsin Inhibitor, 0.1% BSA (TNS, C-41110)
- Collagen Type I Solution (Rat Tail) (We strongly recommend Corning® Inc., product number 354236
- 6.5 mm of Transwell® inserts, 0.4 µm pore size, tissue culture treated polyester membrane polystyrene plates (we strongly recommend Costar® from Corning® Inc., product number 3470-Clear), alternative products see Material List on page 10)

*Use aseptic techniques and a laminar flow bench.*

## II. Protocol

On the day of use, coat the permeable cell culture inserts with collagen type I and seed the HBEpC on the coated inserts. We strongly recommend the use of ALI pre-screened HBEpC in early passages (P3) which results in high TEER-values. Passages >3 may result in a decrease of barrier formation indicated by lower TEER-values

1

### Coating of Transwell® inserts collagen type I solution and cell seeding

Dilute collagen type I stock solution to a working concentration of 0.03 mg/ ml. For 6.5 mm permeable cell culture inserts use 100 µl of collagen type I solution per insert (area of insert = 0.33 $cm^2$). For each 24-well plate a minimum collagen type I working solution of 1.2 ml is needed.

**Example:**

$$\frac{\text{2 ml x 0.03 mg/ml}}{\text{3.9 mg/ml collagen stock solution}} = \textbf{0.015 ml of stock}$$

Add 0.015 ml of collagen type I stock solution to 1.985 ml of PBS in a 15 ml conical tube and mix. Coat each Transwell® insert (the upper chamber only) with 100 µl of the collagen working concentration. For optimal collagen distribution, gently rock the plate from side to side and front to back. Do not swirl the plate. Incubate the plate for 45 minutes in an incubator (37°C, 5% $CO_2$). Carefully aspirate collagen solution from the inserts*. Immediately wash the inserts with 150 µl of PBS. If you want to take a break, keep the PBS on the inserts and store the plate at 37°C.

**Aspiration of inserts can be performed much more easily if you stick a 1,000 µl pipette tip on top of the aspiration pipette. This gives you better control and makes it easier to handle the tiny inserts. Be careful not to damage the membrane with the pipette tip.*

**Note:** We cannot guarantee the barrier-forming function of HBEpC if a commercially available permeable cell culture insert other than Transwell® from Corning or from CELLTREAT® Scientific Products is used. Different commercial Collagen solutions have been qualified for this application (see Material List on page 11). Collagen stock solution should be stored at 2 – 8°C. Let the stock solution acclimate to room temperature (20 – 25°C) before diluting the working concentration. Cold collagen solution is much more viscous and therefore more difficult to pipette. Depeding on the design of your experiment, remember to include one collagen-coated permeable cell culture insert as a „blank" without cells.

2

## Wash the cells

Approximately 5 – 7 days after thawing, the HBEpC should reach 70 – 90% confluence. Aspirate the medium and wash the cells by adding an equal volume of PBS w/o $Ca^{++}/Mg^{++}$.

**Note:** Allow the PBS w/o $Ca^{++}/Mg^{++}$ to reach room temperature before adding to the cells.

3

## Detach the cells

Aspirate the PBS w/o $Ca^{++}/Mg^{++}$ from the vessel and add prewarmed Trypsin/EDTA (100 µl/$cm^2$) to the cells. Gently swirl the vessel to ensure that the cells are completely covered with Trypsin/EDTA. Place the vessel in an incubator (37°C, 5% $CO_2$) for 4 minutes. Check detachment under a microscope. The cells should be rounded. To encourage detachment, you can gently tap the vessel horizontally against the tabletop. Return the vessel to the laminar flow bench and add an equal amount of TNS to the cells. Gently swirl the vessel.

**Note:** Epithelial cells stick tightly to plastic because of the large number of adherens junctions. If the cells do not round after 4 minutes of incubation at 37°C, you can place the vessel in the incubator for 1 additional minute. Do not over-trypsinize them. If they are still sticking after 1 minute of incubation, use a 1,000 µl pipette to wash them down.

4

## Collect the cells and determine the cell number and viability

Transfer the cell suspension to a 15 ml conical tube. To collect all remaining cells, add complete Airway Epithelial Cell Growth Medium to the vessel and transfer into the same 15 ml conical tube. Examine the vessel under a microscope to check if all cells have been collected. Use an appropriate volume of detached cell suspension for determining the cell number. Use your standard methods for cell counting and viability assessment. Spin down the cells (3 minutes at 300 x g) and aspirate the supernatant. Transfer Airway Epithelial Cell Growth Medium to the pellet and resuspend the cells by pipetting them up and down. Keep the cells under the laminar flow bench until you seed them.

5

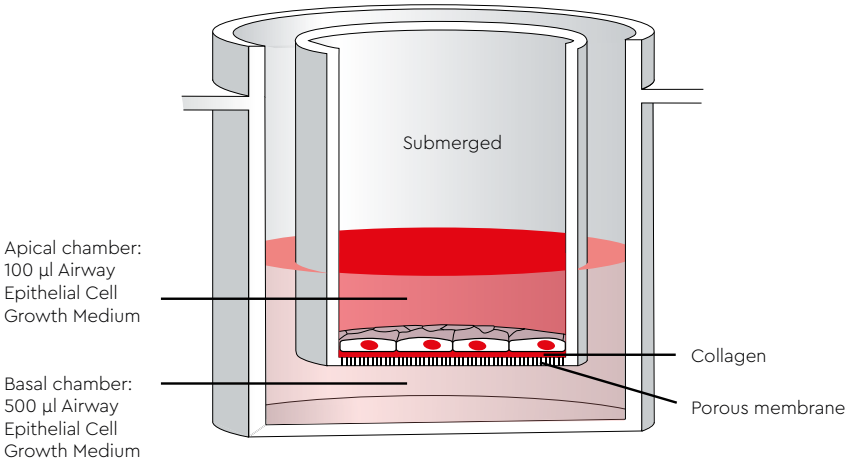
## Plate the cells on collagen type I coated permeable cell culture inserts (24-well plate)

Make sure that the inserts of the multiwell plate have been collagen-coated for at least 45 minutes at 37°C in an incubator, according to step 1. Aspirate the collagen solution from the inserts and wash each insert with 150 µl of prewarmed PBS w/o $Ca^{++}/Mg^{++}$. To avoid evaporation, you can fill the outer wells of the plate with 200 µl PBS (optional). After removing the PBS w/o $CA^{++}/Mg^{++}$ to seed the cells, work quickly to avoid dryness of the semipermeable membrane. After cell counting calculate the desired number of cells. For a 6.5 mm permeable cell culture insert (24-well plate) use a seeding density of 150,000 cells/$cm^2$. The volume of the apical chamber is 100 µl (500,000 living cells/ml). Mix with an appropriate volume of Airway Epithelial Cell Growth Medium for a final concentration of 500,000 cells/ml in a conical tube. For one 6.5 mm Transwell® plate, you need at least 600,000 living cells. If you plan to do TEER measurements, remember to keep one insert as a blank without cells. Transfer 500 µl of Airway Epithelial Cell Growth Medium to the insert in each basal chamber. Afterwards use a 1,000 µl pipette to transfer 100 µl cell suspension into each upper chamber. If you use a blank insert, use 500 µl of Airway Epithelial Cell Growth Medium in the lower chamber and 100 µl of Airway Epithelial Cell Growth Medium in the upper chamber (see Figure 3). For optimal distribution of the cells, gently rock the plate from side to side and front to back. Do not swirl the plate.

6

## Expansion of the cells in submerged culture

Change the medium 24 hours after seeding. Hold the plate at an angle and carefully collect the medium from the lower and upper chambers using an aspiration pipette or 1,000 µl pipette. Be careful to avoid any contact of the pipette tip with the cell layer. Transfer 500 µl of Airway Epithelial Cell Growth Medium to the lower chamber and 100 µl of Airway Epithelial Cell Growth Medium to the upper chamber.




Proliferation phase

***Fig. 3: Collagen type I coated permeable cell culture insert submerged cultivated HBEpC.*** *The porous membrane was coated with collagen type I solution. The basal chamber is only filled with Airway Epithelial Cell Growth Medium, whereas the apical chamber is filled with cell suspension. Cells will attach to the collagen coated membrane after a few hours.*

# Air-Liquid Interface Culture System Protocol Part C

Differentiation of HBEpC at air-liquid interface

This section describes 3D culture of HBEpC on permeable cell culture inserts cultivated in ALI-Airway medium to promote differentiation for 3 – 4 weeks while exposed to air.

## I. Materials

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

*Use aseptic techniques and a laminar flow bench.*

## II. Preparation of ALI medium

The PromoCell Air-Liquid Interface Medium (ALI-Airway) is designed for differentiating plated ALI pre-screened HBEpC on permeable cell culture inserts under airlift conditions. It does not contain adherence factors, so it is mandatory to use it 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. Transfer all supplements to the 500 ml bottle of Basal Medium. Close the bottle and swirl gently until a homogenous mixture is formed. We recommend the addition of 50 µg/ml of Gentamicin (C-42060) for long-term cultivation, especially if you want to perform TEER measurements with an electrode pair. After adding the SupplementMix, the shelf life of the ALI-Airway medium is about 4 weeks. Store the complete growth medium at 2 – 8°C. Do not prewarm the bottle at 37°C. At the time of use, allow the medium to warm up to room temperature. ALI-Airway medium contains light-sensitive components and we therefore recommend protecting it from light.

1

## Initiate the differentiation and start the airlift culture

The cells should be 100% confluent 3 – 4 days after seeding on permeable cell culture inserts (see Figure 4). Check the confluence under a microscope. Carefully aspirate the Airway Epithelial Cell Growth Medium in the lower and upper chambers. Transfer 500 µl of ALI-Airway medium to the lower chamber. Do not pipette any medium into upper chamber. The upper chamber should remain empty – air exposure will stimulate the differentiation process (see Figure 5).

**Note:** Make a timetable for your ALI experiment to prevent problems over the weekend (e.g. thaw cells on Tuesday – passage cells on Monday following week and seed on inserts – change medium on Tuesday – change to ALI-Airway medium on Thursday and airlift the cultures). If the cells do not reach 100% confluence on inserts, change medium and let them grow for another day. It is important for the cell layer to be completely closed when airlifting.

2

## Cultivate the cells under airlift conditions

Replace the ALI-Airway medium in the lower chamber every 2 – 3 days (e.g. Mon-Wed-Fri). On Mondays, change the medium in the morning, on Fridays in the evening. The intact cell layer will prevent diffusion of medium from the lower to the upper chamber. If some medium diffuses to the insert it should be removed. Wash the upper chamber once a week with 150 µl of prewarmed PBS w/o $Ca^{++}$/$Mg^{++}$. Carefully aspirate the PBS without damaging the cell layer. Damaging the cell layer will disrupt the epithelial barrier.

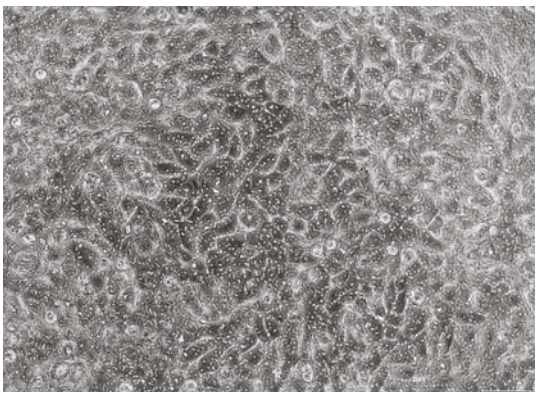


***Fig. 4: HBEpC morphology in submerged culture on permeable cell culture inserts.*** *Image taken on day 4 after seeding HBEpC on Transwell® inserts (Corning®, Inc. article # 3470-clear) in Airway Epithelial Cell Growth Medium. Cells reached confluence in submerged culture, and differentiation can be initiated by ALI-Airway medium and starting the air-liquid culture (10x magnification).*

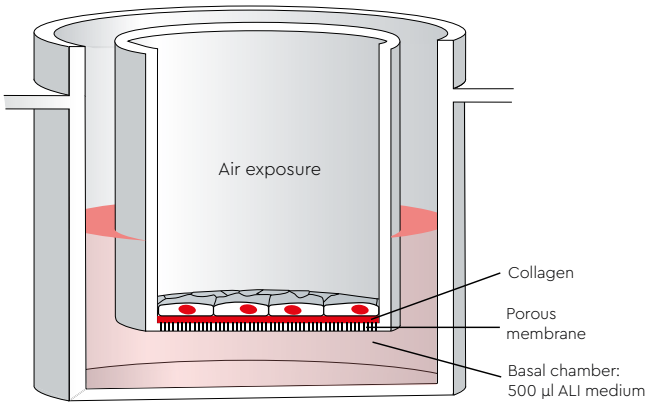



***Fig. 5: Illustration of collagen type I coated permeable cell culture inserts with air exposure of the epithelial layer.*** *During the 3D culture differentiation phase, the ALI-Airway medium is only filled in the basal chamber. Here, the medium is changed every 2 – 3 days whereas the apical chamber is only washed with PBS once a week.*

3

## Differentiation until week 4

We guarantee TEER values >500 Ω*$cm^2$ if you are using ALI pre-screened HBEpC and follow these instructions. Differentiation will be completed on day 28 after airlifting.

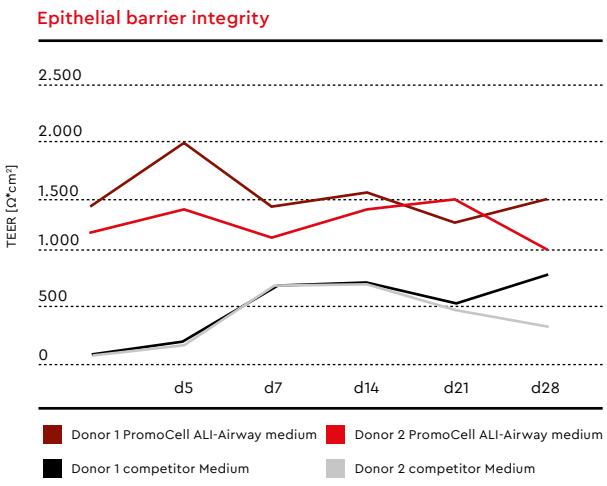
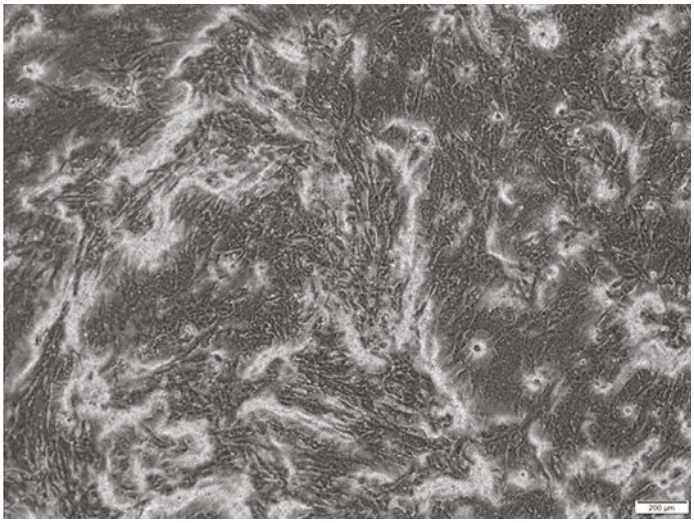



***Fig. 6: TEER values of barrier forming HBEpC over a culture period of 28 days post-airlift.*** *TEER measurement was performed using EVOM2® and STX2 Chopstick® Electrode Set (World Precision Instruments®). Two different HBEpC pre-screened donors (Donor 1 and Donor 2) were compared (6.5 mm Transwell® insert from Corning®, Inc. article # 3470-clear). A competitor ALI medium shows lower TEER values compared to the standardized PromoCell ALI system. The barrier-forming function of PromoCell ALI-Airway medium results in an earlier rise of the TEER values (1st week). This quick rise of the TEER values makes it possible to analyze the epithelial barrier much earlier. Our ALI-Airway medium ensures stable TEER values over 500 Ω*$cm^2$ for different endpoint measurements.*

A



B

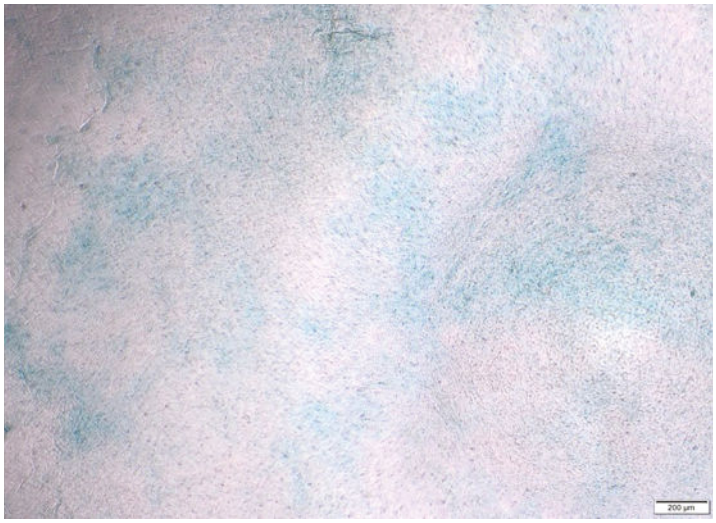
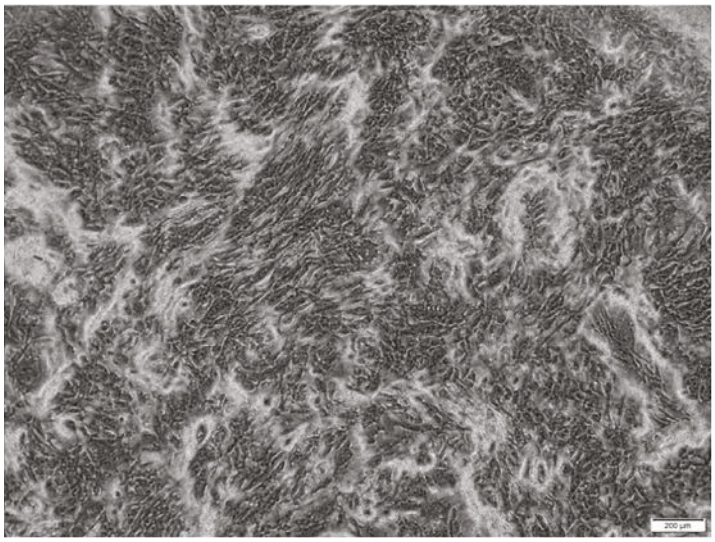


***Fig. 7: Mucin production indicates differentiation of HBEpC cultured under air-liquid conditions. A****, Microscopy of unfixed HBEpC after culturing in ALI-Airway medium for 33 days* ***(6.5 mm Transwell® insert from Corning®, Inc. article # 3470-clear). B****, Alcian Blue staining of fixed cells 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)*

A



B

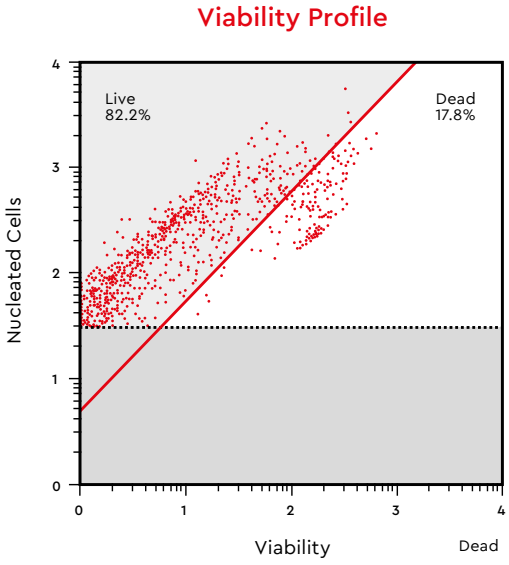



***Fig. 8: After 4 weeks of airlift culture HBEpC show typical morphology and high viability.*** *Cells were cultured in ALI-Airway medium (6.5 mm Transwell® insert from Corning®, Inc. article # 3470-clear) while changing the medium in the basal chamber on Mondays, Wednesdays and Fridays. Once a week, cells were washed with PBS. At this time TEER measurements should be >500 Ω*cm² indicating a monolayer with high integrity and barrier function.* ***A,*** *Microscopy of Transwell® inserts shows an intact cell layer of HBEpC (4x magnification, scale bar 200 µm).* ***B,*** *Cell viability was determined by MUSE Cell Analyzer (Merck Millipore®); and the viability of cells should be >70%.*

# Air-Liquid Interface Culture System Protocol Part D

TEER measurement with an electrode pair

This section describes a method for studying the formation of tight epithelial barriers by transepithelial electrical resistance (TEER) measurement.

## I. Materials

- EVOM® voltohmmeter (World Precision Instruments®)
- STX® Electrode Set (World Precision Instruments®)
- 1.000 Ω test resistor (World Precision Instruments®)

*Use aseptic techniques and a laminar flow bench.*

## II. Equipment

If you wish to measure TEER at different times, always use aseptic techniques and a laminar flow bench. Before you start using the volt-ohmmeter, test it by connecting it to a 1,000 Ω test resistor. Place the STX Chopstick® Electrode Set under laminar flow bench and rinse with 70% EtOH. Let the electrode air dry under the laminar flow bench. Afterwards rinse the electrode 2x with pre-warmed PBS w/o $Ca^{++}/Mg^{++}$. The electrode can be stored for a short time in PBS w/o $Ca^{++}/Mg^{++}$.

1

### Replace the medium in both chambers

Aspirate ALI-Airway medium in the lower chamber and transfer 500 µl prewarmed ALI-Airway medium to the lower chamber and 100 µl medium into upper chamber. Incubate the plate in an incubator (37°C, 5% $CO_2$) for 30 minutes.

2

### Measurement of epithelial resistance with EVOM3® epithelial voltohmmeter

Place the multiwell plate under a laminar flow bench. Rinse the electrode pair of the equibrilated EVOM® twice with pre-warmed PBS w/o $Ca^{++}/Mg^{++}$. Immerse the electrode pair in the „blank" insert (without cells). It is very important for the shorter electrode to be in the upper chamber (see Fig. 10). The electrical resistance of 6.5 mm insert without cells is typically between 150 and 350 Ω. Measure the electrical resistance of your samples.

**Note:** It is not necessary to rinse the electrode between wells. Work fast to avoid changes in the temperature of the culture medium.

3

### Replace the medium in both chambers

The integrity of the epithelial barrier can be measured by the electrical resistance calculated based on Ohm's law of the ratio of voltage and current. To avoid damage of the cells, when applying direct current (DC) voltage an alternating current (AC) voltage signal with a square waveform is also applied (a square wave at a frequency of 12.5 Hz). The STX Chopstick® Electrode Set actually consists of an outer and an inner electrode. The outer electrode is the "current electrode" and the inner electrode is the "voltage electrode". The current electrode passes current through the cell layer on the semipermeable membrane, and the voltage electrode acts as a voltage sensor. The unit of resistance is Ω. To calculate the tissue resistance, the blank resistance (membrane without cells) must be measured and subtracted from the total resistance:

$$R_{Tissue}\ [\Omega] = R_{Total} - R_{Blank}$$

The electrical resistance is inversely proportional to the area of the semipermeable membrane. The TEER values are typically reported as unit area resistance [Ω*$cm^2$].
Resistance of a unit area = resistance [Ω] x effective membrane area [$cm^2$]
Unit area = 1 $cm^2$
Effective membrane area = see permeable cell culture insert product information from manufacturer

**Example:** 6.5 mm Transwell® Corning® Inc., product number 3470 (membrane area = 0.33 $cm^2$), alternative CELLTREAT® 6.5 mm permeable cell culture insert, product number 230635 (membrane area = 0.33 $cm^2$)

$R_{Blank}$ = **140 Ω**
$R_{Total}$ = **2.500 Ω**
$R_{Tissue}$ = **2.360 Ω**
$R_{Reported}$ = **779 Ω*$cm^2$**

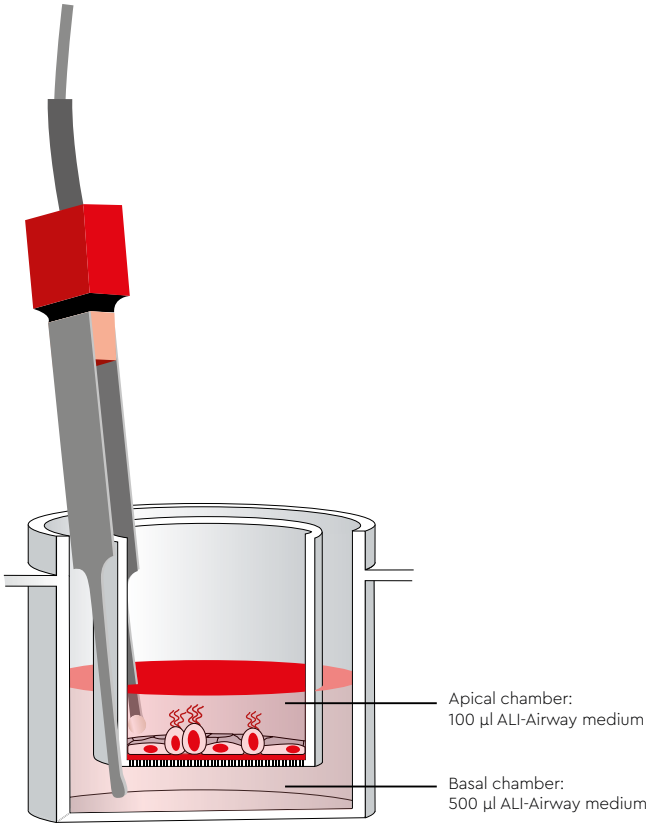



***Fig. 9: Measuring the transepithelial electrical resistance with epithelial voltohmmeter and STX electrode set.*** *Note that the shorter electrode must be placed in the upper chamber and the longer electrode in the lower chamber. Variance in depth of the electrode position must be prevented by allowing the longer electrode to touch the bottom of the dish. Place the electrode pair right angles to the chamber. Do not touch the cell layer.*

## Trademark References

Corning®, Costar® and Transwell® are registered trademarks of Corning® Incorporated. EVOM® and STX Chopstick® Electrode Set are registered trademarks of World Precision Instruments®.

## Material

| Product | Size | Catalog Number |
|---|---|---|
| ALI pre-screened Human Bronchial Epithelial Cells (HBEpC) | 500,000 cryopreserved cells | C-12640 |
| Airway Epithelial Cell Growth Medium (Ready-to-use) | 500 ml | C-21060 |
| Airway Epithelial Cell Growth Medium Kit | 500 ml | C-21160 |
| Air-Liquid Interface Medium (ALI-Airway) | 500 ml | C-21080 |
| Phosphate Buffered Saline w/o $Ca^{++}$/$Mg^{++}$ | 500 ml | C-40232 |
| Trypsin/EDTA (0.04% (w/v) Trypsin/0.03% (w/v) EDTA) | 125 ml | C-41010 |
| Trypsin Neutralizing Solution (0.05% (w/v) Trypsin Inhibitor, 0.1% (w/v) BSA) | 125 ml | C-41110 |

# Additional products strongly recommended for ALI culture

| Coating | Manufacturer | Catalog number | Stock solution* | Working concentration | Volume per insert of a 24-well plate (0,33 $cm^2$ area) | Collagen per serving area |
|---|---|---|---|---|---|---|
| Collagen Type I from rat tail | Corning®Inc. | 354236 | 3.9 mg/ml | 30 µg/ml | 100 µl | 9 µg/$cm^2$ |
| Collagen Type I from rat tail | Sigma-Aldrich® | C3867-1VL | 3.4 mg/ml | 30 µg/ml | 110–277 µl | 10–25 µg/$cm^2$ |
| Collagen Type I from rat tail | Gibco® | A1048301 | 3 mg/ml | 30 µg/ml | 55–100 µl | 5–9 µg/$cm^2$ |
| Collagen Type IV from human placenta | Sigma-Aldrich® | C5533-5MG | 1 mg/ml | 30 µg/ml | 110–330 µl | 10–30 µg/$cm^2$ |
| PureCol® Bovine Collagen | CellSystems® | 5005-100 ML | 3 mg/ml | 30 µg/ml | 66–198 µl | 10–30 µg/$cm^2$ |

* *Concentrations may vary depending on different batches*

***Table 1:*** *Overview of tested collagen coatings for Air-Liquid Interface culture using our ALI pre-screened Human Bronchial Epithelial cells and Air-Liquid Interface Medium (ALI-Airway). All working concentrations were diluted in PBS w/o Ca++ Mg++ and coating for porous membranes was performed for one hour at room temperature.*

| Product | Size | Catalog Number |
|---|---|---|
| Collagen Type I (Rat Tail) (Corning®) | 3 – 4 mg/ml | 354236 |
| Permeable Cell Culture Inserts Packed in 24 Well Plate, 0.4 µm PET (CELLTREAT® Scientific Products) | - | 230635 |
| Costar® Transwell® (24 well plate) (Corning®) | - | 3470 |
| EVOM® (World Precision Instruments®) | - | - |
| STX® Electrode Set (World Precision Instruments®) | - | - |
| 1.000 Ω test resistor (World Precision Instruments®) | - | 91750 |

# 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): p. L25-L31.

**2.** Lacroix, G., et al., Air–liquid Interface in vitro models for respiratory toxicology research: consensus workshop and recommendations. Applied in vitro toxicology, 2018. 4(2): p. 91–106.

**3.** Knowles, M.R., et al., Measurements of transepithelial electric potential differences in the trachea and bronchi of human subjects in vivo. American Review of Respiratory Disease, 1982. 126(1): p. 108–112.

**4.** Fish, R.M. and L.A. Geddes, Conduction of electrical current to and through the human body: a review. Eplasty, 2009. 9.

**5.** Bhat, M., et al., Regulation of tight junction permeability by calcium mediators and cell cytoskeleton in rabbit tracheal epithelium. Pharmaceutical research, 1993. 10(7): p. 991–997.

**6.** Srinivasan, B., et al., TEER measurement techniques for in vitro barrier model systems. Journal of laboratory automation, 2015. 20(2): p. 107–126.

**7.** Barar, J., A. Maleki, and Y. Omidi, Modulation of cellular transport characteristics of the human lung alveolar epithelia. Iranian Journal of Pharmaceutical Research, 2010: p. 163–171.

**8.** Fulcher, M.L., et al., Well-differentiated human airway epithelial cell cultures, in Human cell culture protocols. 2005, Springer. p. 183–206.

**9.** Spina, D., Epithelium smooth muscle regulation and interactions. American journal of respiratory and critical care medicine, 1998. 158(supplement_2): p. S141-S145.

**10.** Harkema, J., et al., Epithelial cells of the conducting airways: a species comparison. Lung biology in health and disease, 1991. 55: p. 3–39.

**11.** Pilewski, J.M. and R.A. Frizzell, Role of CFTR in airway disease. Physiological reviews, 1999. 79(1): p. S215-S255.

**12.** Inayama, Y., et al., In vitro and in vivo growth and differentiation of clones of tracheal basal cells. The American journal of pathology, 1989. 134(3): p. 539.

**13.** Ayers, M. and P. Jeffery, Proliferation and differentiation in mammalian airway epithelium. European Respiratory Journal, 1988. 1(1): p. 58–80.

**14.** Ma, J., B.K. Rubin, and J.A. Voynow, Mucins, mucus, and goblet cells. Chest, 2018. 154(1): p. 169–176.

**15.** Lumsden, A.B., A. McLean, and D. Lamb, Goblet and Clara cells of human distal airways: evidence for smoking induced changes in their numbers. Thorax, 1984. 39(11): p. 844–849.

**16.** Hicks Jr, W., et al., Isolation and characterization of basal cells from human upper respiratory epithelium. Experimental cell research, 1997. 237(2): p. 357–363.

**17.** Hajj, R., et al., Basal cells of the human adult airway surface epithelium retain transit amplifying cell properties. Stem Cells, 2007. 25(1): p. 139–148.

**18.** Brechbuhl, H.M., et al., β-catenin dosage is a critical determinant of tracheal basal cell fate determination. The American journal of pathology, 2011. 179(1): p. 367–379.

**19.** Evans, M.J., et al., The role of basal cells in attachment of columnar cells to the basal lamina of the trachea. Am J Respir Cell Mol Biol, 1989. 1(6): p. 463–469.

**20.** Ganesan, S., A.T. Comstock, and U.S. Sajjan, Barrier function of airway tract epithelium. Tissue barriers, 2013. 1(4): p. e24997.

**21.** Knight, D.A. and S.T. Holgate, The airway epithelium: structural and functional properties in health and disease. Respirology, 2003. 8(4): p. 432–446.

**22.** Weiser, N., et al., Paracellular permeability of bronchial epithelium is controlled by CFTR. Cellular Physiology and Biochemistry, 2011. 28(2): p. 289–296.

**23.** Knowles, M., et al., Bioelectric properties and ion flow across excised human bronchi. Journal of Applied Physiology, 1984. 56(4): p. 868–877.

**24.** Rezaee, F. and S.N. Georas, Breaking barriers. New insights into airway epithelial barrier function in health and disease. American journal of respiratory cell and molecular biology, 2014. 50(5): p. 857–869.



**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





06/2023