Air-Liquid Interface Medium (ALI-Airway)

PromoCell®

Instruction Manual

Product	Size	Catalog Number
Air-Liquid Interface Medium (ALI-Airway)	500 ml	C-21080

Recommended for

- C-12640 HBEpC (Primary Human Bronchial Epithelial Cells, ALI pre- screened)

Recommended products

- C-21060 Airway Epithelial Cell Growth Medium (Ready-to-use)
- C-41210 DetachKit

Product Description

Our Air-Liquid Interface Medium (ALI-Airway) (C-21080) is a serum- and BPE-free formulation for an optimal and standardized culture of Human Bronchial Epithelial Cells (HBEpC) at the air-liquid interface (ALI). The medium comes in a ready-to-use format containing the Basal Medium together with a SupplementMix. Primary HBEpCs cultured in vitro in PromoCell Air-Liquid Interface Medium fastly develop an epithelial barrier formed by tight junctions. Thereby, the cells stay >70% viable for at least 14 days in culture and a stable transepithelial electrical resistance (TEER) of >500 Ω*cm² is measurable over the culture period. The maintenance of high TEER values is a strong indicator for an optimal epithelial barrier which is formed by tight junctions.

In addition to the ALI-Airway medium, we offer primary HBEpCs that are pre-screened for ALI cultures. Together with our Airway Epithelial Cell Growth Medium (C-21060) and ALI pre-screened HBEpC (C-12640), PromoCell Air-Liquid Interface Medium (C-21080) constitute a standardized, serum- and BPE-free culture system for a human airway model to study respiratory biology, airway infection, and airway disease.

Note: The ALI-Airway medium is not intended for the expansion of primary epithelial cells since it contains only low levels of growth factors. For expansion phase we recommend our Airway Epithelial Cell Growth Medium (C-21060).

Supplementation Details

The ready-to-use SupplementMix is serum- and BPE-free containing all necessary growth factors and supplements.

Note: The Air-Liquid Interface Medium does not contain antibiotics or antimycotics and is formulated for use in an incubator with an atmosphere of 5% CO_2.

Preparation of the Supplemented Medium for Use

Thaw the SupplementMix at 15 to 25°C. Aseptically mix the supplement solution by carefully pipetting up and down. Then, transfer the entire content of the SupplementMix to the Basal Medium. Close the bottle and swirl gently until a homogenous mixture is formed.

Storage and Stability

Store the Basal Medium at 2 to 8°C in the dark and the SupplementMix at -20°C immediately after arrival. Do not freeze the Basal Medium. If stored properly, the products are stable until the expiry date stated on the label. After adding the SupplementMix to the Basal Medium, the shelf life of the complete medium is 6 weeks at 2 to 8°C. Do not freeze the complete medium. For use, pre-warm only an aliquot of the complete medium and keep the remaining medium refrigerated at 2 to 8°C.

Quality Control

All lots and components of PromoCell Air-Liquid Interface Medium are subjected to comprehensive quality control tests using ALI pre-screened HBEpC by analyzing the transepithelial electrical resistance (TEER) of the cells over a time period of at least 14 days and TEER values >500 Ω*cm².

In addition, all lots of media have been tested for the absence of microbial contaminants (fungi, bacteria, mycoplasma).

Intended Use

The products are for in vitro use only and not for diagnostic or therapeutic procedures. For safety precautions please see appropriate MSDS.

Follow appropriate safety precautions!

The Air-Liquid Interface Medium contains light sensitive components. Avoid long exposure times to light, e.g. store in a cabinet when warming the medium to room temperature.

Air-Liquid Interface Culture

This protocol describes the generation of a stable 3D human airway model with primary human bronchial epithelial cells using our Air-Liquid Interface Culture System.

I. Submerged culture on collagen type I coated permeable cell culture inserts

Materials

- C-12640 ALI pre-screened Human Bronchial Epithelial Cells
- C-21060 Airway Epithelial Cell Growth Medium (Ready-to-use)
- C-21080 Air-Liquid Interface Medium (ALI-Airway)
- 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)
- alternative CELLTREAT® Permeable Cell Culture Inserts Packed in 24-well Plate, 0.4 µm PET can be used from CELLTREAT Scientific Products, product number 230635)
- Collagen Solution (we recommend Collagen Type I Solution from rat tail Corning®Inc., product number 354236, however, please find a list of tested coatings and concentrations on page 4, table 1)
- C-40232 Phosphate Buffered Saline w/o Ca^{++}/Mg^{++}
- C-41010 Trypsin/EDTA (0,04 % Trypsin/0.03 % EDTA)
- C-41110 TNS (0.05 % Trypsin Inhibitor, 0.1 % BSA)
- Gentamicin-Sulfate solution with a final concentration of 50 µg/ml in the medium

Use aseptic techniques and a laminar flow bench.

1

Expansion of ALI pre-screened HBEpC in Airway Epithelial Cell Growth Medium

Thaw and expand ALI pre-screened Human Bronchial Epithelial Cells (HBEpC) using PromoCell Airway Epithelial Cell Growth Medium (Ready-to-use) according to the corresponding instruction manual for the handling of epithelial cells. The instruction manual can be downloaded on our website www.promocell.com.

Note: The best barrier formation for ALI culture using ALI pre-screened HBEpC can be observed 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.

2

Collagen type I coating of permeable cell culture inserts

We strongly recommend using collagen type I for coating the permeable cell culture inserts on the day of use. Coat the culture vessel with collagen according to the instruction manual of
the collagen solution. In table 1 below you can find a list of tested collagen solutions and working concentrations.
For a detailed instruction about collagen coating of the insers please refer to our Application Note *Air-Liquid Interface Culture System for Standardized Respiratory Research*. The Application Note can be downloaded on our website **www.promocell.com**

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 4). 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.

3

Detach the expanded HBEpC

When the HBEpC are grown to 70 to 80% confluency, aspirate the medium and wash the cells by adding an equal volume of PBS w/o Ca^{++}/Mg^{++} at room temperature. 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. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.

4

Neutralize the trypsin and determine the cell number and viability

Add 100 µl Trypsin Neutralization Solution per cm^2 of vessel surface and gently agitate. Carefully aspirate the cell suspension and transfer it to a centrifugation tube. 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 for 3 minutes at 300 x g and aspirate the supernatant. Transfer PromoCell 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

Re-plate and expand the cells on collagen coated permeable cell culture inserts

After cell counting calculate the required number of cells. For a 6.5 mm inserts (24-well plate) use a seeding density of 150,000 cells/cm^2.

Transfer 500 µl of Airway Epithelial Cell Growth Medium in each basal chamber of the Transwell®. Afterwards use a 1,000 µl pipette to transfer the corresponding cell suspension into each upper chamber (with a 100 µl volume). 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. For optimal distribution of the cells, gently rock the plate from side to side and front to back. Do not swirl the plate. Place the vessel in an incubator (37°C, 5% CO_2) for cell attachment.

Change the medium 24 hours after seeding by adding 500 µl Airway Epithelial Cell Growth Medium to the lower chamber and 100 µl of Airway Epithelial Cell Growth Medium to the upper chamber.

II. Differentiation on air-liquid interface

See page 2.

Use aseptic techniques and a laminar flow bench.

1

Initiate the differentiation

The cells should be 100% confluent 3 to 4 days after seeding them on Transwell®. In order to achieve a proper barrier function, it is recommended to perform the airlift after five days of submerged culture in Airway Epithelial Cell Growth Medium. Carefully aspirate the Airway Epithelial Cell Growth Medium from the lower and upper chambers. Transfer 500 µl of ALI-Airway medium to the lower chamber. Do not add any medium into upper chamber. The upper chamber should remain empty as the air exposure will stimulate the differentiation process.

Note: It is important for the cell layer to be completely confluent when airlifting.

2

3D cultivation at airlift

Replace the ALI-Airway medium in the lower chamber every 2 to 3 days. 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.

We guarantee TEER values >500 Ω*cm^2 if you are using ALI pre-screened HBEpC and follow these instructions.

Coating	Manufacturer	Catalog Number	Stock Solution*	Working Concentration	Volume per Insert of a 24-well plate (0,33 cm^2 area)	Collagen Coating per surface 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	50 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 of porous membranes was performed for one hour at room temperature.*

If you require special media modifications, we offer a custom media service starting at 10 bottles per order.
Contact us at info@promocell.com to find out more.

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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