Air-Liquid Interface (ALI) Differentiation Protocol for Human Bronchial Epithelial Cells

propagenix

Description

The below procedure outlines the Air-Liquid Interface (ALI) differentiation protocol for bronchial epithelial cells expanded in EpiX™ medium. Please follow the EpiX Epithelial Cell Expansion Protocol to generate the required number of cells for differentiation. Harvest the bronchial epithelial cells when they approach 80 – 90% confluence and seed them on porous membrane insert at high density. During the next few days, culture the cells by adding medium to both bottom and apical chambers and allow the cells grow to complete confluence. Then remove the medium from the apical chamber and feed the cells only from the bottom chamber, continue to culture the cells at the air-liquid interface for 3–4 weeks to promote differentiation.

Intended Use

For Research Use Only.

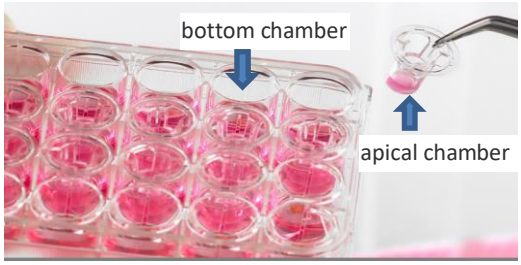
Reagents required

1. 2M $CaCl_2$, e.g., Quality Biological, Cat# 351-130-721
2. PneumaCult-ALI medium, StemCell Technologies, Cat# 05001
3. Polyester Transwell insert, e.g., Transwell® Inserts, Corning, Cat# 3470
4. Cell Culture Companion Plates for Inserts, e.g., Corning, Cat# 353504
5. Ca^{2+}, Mg^{2+} free PBS, e.g., Thermo Fisher Scientific, Cat# 10010-023
6. EVOM2, Epithelial Volt/Ohm Meter for TEER measurement, World precision instruments.
7. ENDOHM-6 (EndOhm for 6mm culture cups), World precision instruments.

Procedure

1. Prepare **Modified EpiX™ medium** for submerge phase culture. Add extra $CaCl_2$ to bring the final concentration of $CaCl_2$ to 1.5 mM, for example, add 75 µl 2M $CaCl_2$ to 100 ml EpiX™ medium.
2. **Medium for ALI phase: PneumaCult-ALI medium or equivalent.** Prepare PneumaCult-ALI medium following the instruction provided by the supplier.

   For a 24-well Transwell insert, use 200–250 µl medium in the apical chamber, and 800 µl–1 ml medium in the bottom chamber.

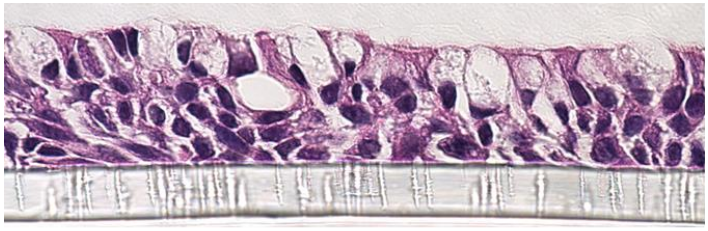


3. Pre-wet the membrane by adding 300 µl PBS to the apical chamber. Carefully aspirate the PBS after 5–10 minutes without touching the membrane.
4. Harvest bronchial epithelial cells as described in Section III above, and count cell numbers.
5. Dilute the cells in **Modified EpiX™ medium** to 500,000 cells/ml, and add 250 µl cell suspension (i.e., 125,000 cells per 24-well insert, which is about 400,000 cells/cm^2) to the apical chamber. Add 800 µl–1 ml **Modified EpiX™ medium** to the bottom chamber.
6. Culture the cells at 37 °C in an incubator with 5% CO_2. Keep the cells in submerged phase for a few days until the cells form complete monolayer. Change the **Modified EpiX™ medium** in both chambers every other day.

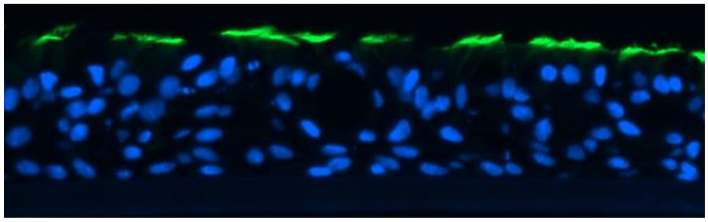
7. After a complete monolayer is formed on Transwell insert, remove the **Modified EpiX™ medium** from both apical and bottom chambers. Add 800 µl–1 ml **PneumaCult-ALI** medium to the bottom chamber only. This marks the beginning (day 0) of air-liquid interface phase. Going forward, do not add media to the apical chamber.
8. Change the **PneumaCult-ALI medium** in the bottom chamber every 2–3 days. Once a week, gently rinse the apical chamber with 300 µl PBS to remove any mucin produced by the cells during the differentiation.
9. If necessary, measure TEER using EVOM2 and EndOhm-6 by following the instructions provided by WPI (https://www.wpiinc.com/clientuploads/pdf/EVOM2_IM.pdf). Use one insert without epithelial cells as blank for the TEER measurement.

Examples of ALI differentiation

Human bronchial epithelial cells are expanded using EpiX™ medium for 8 passages (>25 PDs) and differentiated at air-liquid interface for 21 days. The differentiated epithelium (on Transwell insert) is embedded in paraffin and sectioned at 5 µm. The sections are stained with hematoxylin and eosin (H&E), or a monoclonal antibody against acetylated-tubulin to demonstrate multiciliated cells, or alcian blue to demonstrate mucin-producing cells.



H&E



Anti-acetylated tubulin

Alcian Blue