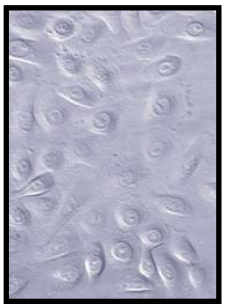
REF: P10557

PULMONARY SYSTEM INNOPROFILE™
HUMAN BRONCHIAL EPITHELIAL CELLS



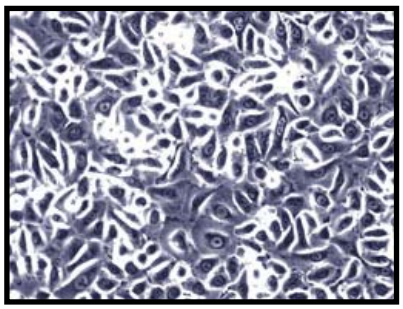
Product Type:	Cryo-preserved Bronchial Epithelial Cells
Catalog Number:	P10557
Source:	Human Bronchi
Number of Cells:	5 x 10^5 Cells / vial (1ml)
Storage:	Liquid Nitrogen

Human Bronchial Epithelial Cells (HBEpiC) provided by Innoprot are isolated from human healthy bronchi. HBEpiC are cryopreserved at passage one and delivered frozen. HBEpiC are guaranteed to further expand for 15 population doublings under the conditions provided in this technical sheet.

The bronchial epithelium consists of the surface epithelial cells and mucus glands. The surface epithelial cells are made up of three principle cell types: basal, goblet, and ciliated cells, of which the latter two form a suprabasal columnar structure and are necessary for mucociliary clearance. Studies using bronchial epithelial cell culture differentiated to induce a mucociliary phenotype have shown that IL-4 and IL-13 stimulation modulate basic cell functions such as proliferation, ciliary beating, and mucous production. Cell proliferation, as well as various other cellular processes in bronchial epithelial cells, is also regulated in part by EGF receptor signalling.

Recommended Medium

- Bronchial Epithelial Cell Medium-Serum Free (Reference: P60151-SF)



Product Characterization

Immunofluorescent method

- Cytokeratin-18
- Cytokeratin-19
- Vimentin

The cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi

Product Use

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures

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INSTRUCTIONS FOR CULTURING CELLS

IMPORTANT: Cryopreserved cells are very delicate. Thaw the vial in a 37 °C waterbath and return them to culture as quickly as possible with minimal handling!

Set up culture after receiving the order:

1. Prepare a collagen type I-coated flask following manufacturer instructions. Leave the flask in incubator overnight (minimum four hours at 37°C incubator).

2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.

3. Rinse the collagen-coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.

4. Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using a 1 ml eppendorf pipette gently re-suspend the contents of the vial.

5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 5,000 cells/cm^2 is recommended.

Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture.
It is also important that smooth muscle cells are plated in poly-L-lysine coated flask that promotes cell attachment and growth.

6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.

7. Return the culture vessels to the incubator.

8. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter.

Maintenance of Culture:

1. Refresh supplemented culture medium the next morning after establishing a culture from cryopreserved cells.

2. Change the medium every three days thereafter, until the culture is approximately 70% confluent.

3. Once the culture reaches 70% confluency, change medium every other day until the culture is approximately 90% confluent. other day until the culture is approximately 90% confluent.

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Subculture:

1. Subculture when the culture reaches 90% confluency.

2. Prepare collagen-coated culture vessels one day before subculture.

3. Warm complete medium, trypsin/EDTA solution (T/E), TNS neutralization solution (TNS), and DPBS (Ca^{++}-and Mg^{++}-free) to room temperature. We do not recommend warming reagents and medium in a 37ºC water bath prior to use.

Note: *DPBS, trypsin/EDTA solution & trypsin neutralization solution are included in the "Primary Cells Detach Kit provided by Innoprot (Cat. Nº P60305).*

4. Rinse the cells with DPBS.

5. Add 8 ml of DPBS and then 2 ml of T/E solution into flask (in the case of a T-75 flask). Gently rock the flask to ensure complete coverage of cells by T/E solution. Incubate the flask in a 37ºC incubator for 1 to 2 minutes or until cells completely round up. Use a microscope to monitor the change in cell morphology.

6. During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum.

7. Transfer T/E solution from the flask to the 50 ml centrifuge tube (a small percent of cells may detach) and continue to incubate the flask at 37ºC for another 1 to 2 minutes (no solution in the flask at this moment).

8. At the end of incubation, gently tap the side of the flask to dislodge cells from the surface. Check under a microscope to make sure that all cells detach.

9. Add 5 ml of TNS solution to the flask and transfer detached cells to the 50 ml centrifuge tube. Rinse the flask with another 5ml of TNS to collect the residual cells.

10. Examine the flask under a microscope for a successful cell harvest by looking at the number of cells being left behind; there should be less than 5%.

11. Centrifuge the 50 ml centrifuge tube at 1000 rpm for 5 minutes. Resuspend cells in culture medium.

12. Count and plate cells in a new collagen-coated culture vessel with the recommended cell density.

Caution: *Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions mush be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].*

[1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. *J Tissue Culture Methods*. 11(4).

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