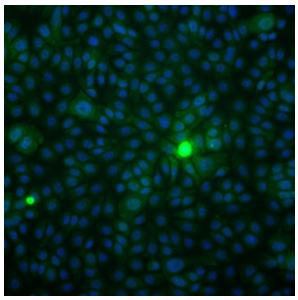
REF: P10557-IM

PULMONARY INNOPROFILE™
IMMORTALIZED HUMAN BRONCHIAL EPITHELIAL CELL LINE



Product Type:	Immortalized Bronchial Epithelial Cell Line
Catalog Number:	P10557-IM
Immortalization:	hTERT
Number of cells:	> 1 x10^6 cells in Cryopreserved vials
Storage:	Liquid Nitrogen

P10557-IM have been obtained immortalizing Human Bronchial Epithelial Cells with Lenti-SV40 Lentivirus. Immortalized cells were controlled passaging side by side with the primary cells. Primary cells go into senescence after the 3rd passage while the SV40-tranduced cells go beyond 30 passages.

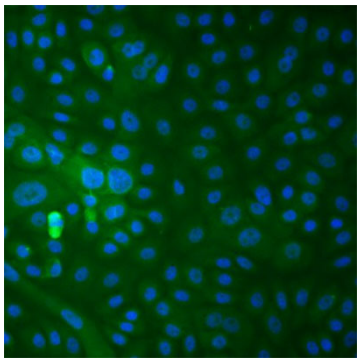
The respiratory epithelium comprises a mixed population of ciliated, nonciliated, and mucous-secreting cells from proximal to distal airways. The individual characteristics of these cells create not only an effective physical barrier against various noxious substances, but also a highly sophisticated host defense system by producing and releasing a large number of chemical mediators and cytokines. The bronchial epithelium consists of both, the surface epithelial cells and mucus glands. The surface epithelial cells comprise three principle cell types; basal, goblet, and ciliated cells, of which the latter two form a suprabasal columnar structure. They are necessary for mucociliary clearance. Cultured BEpiC are a useful in vitro model for studying the function and pathophysiology of the bronchial epithelium.

Product Characterization

Positive for:

- CK-18
- CK-19
- ZO-1

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



Recommended Medium

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

Product Use

Sale of this item is subjected to the completion of a Material Transfer Agreement (MTA) by the purchasing individual/institution for each order. If you have any questions regarding this, please contact us at innoprot@innoprot.com

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INSTRUCTIONS FOR CULTURING CELLS

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

Set up culture after receiving the order:

1. Prepare a collagen-coated culture vessel). Leave the vessel in a 37ºC incubator overnight (or for a minimum of three hours).

2. Prepare complete medium. Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.

3. Rinse the collagen-coated vessel twice with sterile water and then add 15 ml of complete medium. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.

4. Place the frozen vial in a 37ºC water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field.

5. Carefully remove the cap without touching the interior threads. Gently resuspend and dispense the contents of the vial into the equilibrated, collagen-coated culture vessel. A seeding density higher than 7,500 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 residual DMSO in the culture. It is also important that cells are plated in collagen-coated culture vessels to promote cell attachment.

6. Replace the cap or lid of the culture vessel and gently rock the vessel to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.

7. Return the culture vessel to the incubator.

8. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove 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.

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Subculture:

1. Subculture when the culture reaches 90% confluency or above.

2. Prepare collagen-coated culture vessels (2 µg/cm^2) one day or three hours before subculture.

3. Warm complete medium, trypsin/EDTA solution (T/E), Trypsin 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.

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 (FBS).

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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