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IMMORTALIZED CELL LINES

P10556-IM

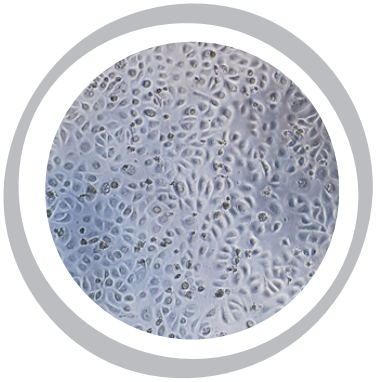
Immortalized Human Pulmonary Alveolar Epithelial Type-II Cells provided by Innoprot have been obtained by immortalizing human pulmonary alveolar epithelial type-II cells with Lenti-SV40 lentivirus.

Pulmonary alveolar epithelial cells line the alveoli of the lungs, playing a critical role in gas exchange and maintaining the integrity of the air-blood barrier. They consist primarily of two major cell types: type I cells, which facilitate gas diffusion, and type II cells, which produce surfactant to reduce surface tension and support alveolar repair.

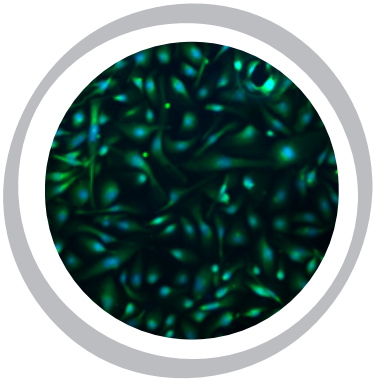
In pathological conditions such as acute lung injury (ALI), chronic obstructive pulmonary disease (COPD), or pulmonary fibrosis, alveolar epithelial dysfunction contributes to inflammation, fibrosis, and impaired oxygenation. Additionally, these cells are involved in immune responses and tissue remodeling, influencing disease progression through signaling pathways such as TGF-β and NF-κB.

IMMORTALIZED HUMAN PULMONARY
ALVEOLAR EPITHELIAL TYPE-II CELLS

Product Type: Immortalized Human Pulmonary Alveolar Epithelial Type-II Cells
Catalog Number: P10556-IM
Immortalization: SV40 Large T Antigen. G418 resistant.
Number of cells: >1x10^6 cells (cryopreserved vials)
Storage: Liquid Nitrogen
Recommended Medium: Alveolar Epithelial Cell Medium (Ref: P60102)
Product Characterization: Expression of constitutive phenotypic epithelial markers (SFTPC and citokeratin).
Functional analysis (angiogenesis reorganization).



Phase-contrast



SFTPC

About Immortalized Human Pulmonary Alveolar Epithelial Cells

The immortalized human pulmonary alveolar epithelial type II cell line has been developed through genetic modification of primary culture of human pulmonary alveolar epithelial type II cells, employing the SV40LT protein as the immortalization method. The SV40LT protein, derived from the Simian Virus 40 Large T-antigen, has been introduced to confer immortality to the cells, allowing for an extended lifespan compared to primary cells that typically undergo senescence after a limited number of passages. The use of SV40LT for immortalization is a common technique in cell biology and allows for the establishment of cell lines with a more stable and prolonged growth capacity. This modification enables researchers to conduct long-term experiments and studies that require consistent cellular behavior over an extended period. Primary cells exhibit senescence following the 5th passage, whereas the SV40LT-transduced cells demonstrate a prolonged viability, extending beyond 20 passages.

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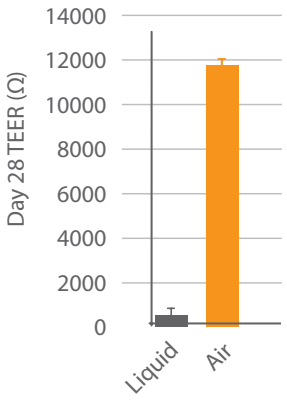
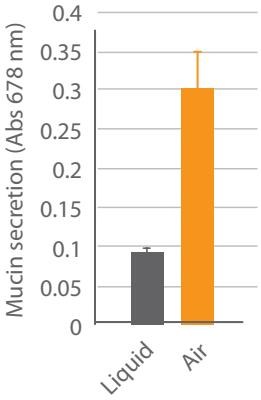
Functional Analysis

1. Measurement of Transepithelial Electrical Resistance (TEER) in Differentiated Cultures
To assess epithelial barrier integrity, 150,000 cells per insert (P24 plate format) are seeded onto collagen-coated membranes in complete culture medium. Medium is added to both the apical and basal compartments of the insert. After two days, once the cells reach confluency and form a monolayer, the differentiation process is initiated. The culture medium is replaced with differentiation medium, and the apical medium is removed to establish an air-liquid interface (ALI). The medium is refreshed twice a week. On day 28, TEER (Ω) measurements are performed. An increase in TEER is indicative of epithelial differentiation and barrier maturation. Cells cultured under ALI conditions exhibit higher transepithelial resistance compared to control cultures, confirming a more differentiated epithelial phenotype.

2. Measurement of Mucin Secretion (Glycosaminoglycans) Using Alcian Blue Staining
In the same differentiated wells from the previous assay, cells are fixed with 4% paraformaldehyde for 15 minutes and stained with 1% Alcian Blue in 0.1M HCl, incubating overnight. After staining, the bound dye is solubilized using 0.1M HCl, and absorbance is measured at 678 nm. Results show that differentiated cells exhibit increased mucin production, confirming enhanced epithelial specialization.





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Culturing conditions

1 IMMEDIATELY UPON DELIVERY

1.1 Remove the vial from the shipping container to check for freezing.

1.2 Transfer the frozen vial to liquid nitrogen until ready to thaw.

2 THAWING CELLS:

2.1 Prepare "Thawing medium" by combining 500 ml of basal medium, 25 ml of fetal bovine serum, 5 ml of Growth supplement and 5 ml of penicillin/streptomycin solution.

2.2 Thaw cells rapidly in a 37°C water bath; avoid allowing the sample to warm to 37°C. Cryovials should be cool to the touch when removed.

2.3 Remove the vial, wipe it dry, and transfer it to a sterile field.

2.4 Rinse the vial with 70% ethanol, then wipe to remove excess. Open the vial and resuspend its contents using a 1 ml Eppendorf pipette.

2.5 Dispense the contents into a 25 cm^2 culture flask with warm complete media (FBS percentage can be increased up to 10% for better culture establishment).

2.6 Place the flask in the incubator.

2.7 For optimal results, avoid disturbing the culture for 16 hours after initiation. Change the growth medium the next day to remove unattached cells, then every other day thereafter.

3 MAINTENANCE OF THE CULTURE:

3.1 Change medium 48 hours after establishing a subculture.

3.2 Subculture when cells are over 90% confluent.

4 SUBCULTURING:

Remove medium, rinse with 0.05% trypsin-EDTA solution. Add 1 to 2 mL of trypsin-EDTA solution and allow the flask to sit until cells detach. Add fresh culture medium, aspirate, and dispense into new culture flasks.

Recommended subcultivation ratio of 1:2 to 1:6.

Medium Renewal: 2 to 3 times per week.

Reagents for cryopreservation: Cryostor S10.

Quality Control / Biosafety

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

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