ATCC® Normal Human Primary Lobar Epithelial Cells, when grown in Airway Epithelial Cell Basal Media supplemented with Bronchial/Tracheal Epithelial Cell Growth Kit components, provide an ideal cell system to propagate bronchial/tracheal epithelial cells in serum free conditions. The cells are cryopreserved at the second passage to ensure the highest viability and plating efficiency. ATCC® Primary Cell Solutions™ cells, media, supplements and reagents are quality tested together to guarantee optimum performance and reliability.

Cell Characteristics

**Tissue:** Respiratory tract

**Morphology:** epithelial, packed cuboidal morphology

**Growth Properties:** adherent

### Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

### SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

### Unpacking & Storage Instructions

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

### Preparation of Complete Growth Medium

One bottle of Airway Epithelial Cell Basal Medium (ATCC PCS-300-030) plus Bronchial Epithelial Cell Growth Kit (ATCC PCS-300-040)

1. Obtain one Bronchial/Tracheal Epithelial Cell Growth Kit from the freezer; make sure that the caps of all components are tight.
2. Thaw the components of the growth kit just prior to adding them to the basal medium. Warm the L-glutamine component in a 37°C water bath and shake to dissolve any precipitates prior to adding to the basal medium.
3. Obtain one bottle of Airway Epithelial Cell Basal Medium (485 mL) from cold storage.
4. Decontaminate the external surfaces of all growth kit component vials and the basal medium bottle by spraying them with 70% ethanol.
5. Using aseptic technique and working in a laminar flow hood or biosafety cabinet, transfer the indicated volume of each growth kit component, as indicated in Table 1, to the bottle of basal medium using a separate sterile pipette for each transfer.

#### Table 1. Bronchial/Tracheal Epithelial Cell Growth Kit Components

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLL Supplement</td>
<td>1.25 mL</td>
<td>HSA 500 mg/mL, Linoelic Acid 0.6 mM, Lecithin 0.8 mg/mL</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>15 mL</td>
<td>6 mM</td>
</tr>
<tr>
<td>Extract P</td>
<td>2.0 mL</td>
<td>0.4%</td>
</tr>
<tr>
<td>Airway Epithelial Cell</td>
<td>5.0 mL</td>
<td>Epinephrine 1.0 mM, Transferrin 5 mg/mL, T3 10 nM, Hydrocortisone 5 mg/mL, rh EGF 5 ng/mL, rh Insulin 5 mg/mL</td>
</tr>
</tbody>
</table>

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: Primary Lobar Epithelial Cells (ATCC® PCS-300-015™)
**Primary Lobar Epithelial Cells (ATCC® PCS-300-015™)**

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Antimicrobials and phenol red are not required for proliferation but may be added if desired. The recommended volume of either of the *optional* components to be added to the complete growth media is summarized in **Table 2**.

**Table 2. Addition of Antimicrobials/Antimycotics and Phenol Red (Optional)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final Concentration</th>
</tr>
</thead>
</table>
| Penicillin-Streptomycin-Amp | 0.5 mL | Penicillin: 10 Units/mL   
| Solution                   |        | Streptomycin: 10 μg/mL    
|                            |        | Ampicillin B: 25 μg/mL    |
| Phenol Red                 | 0.5 mL | 33 μM                |

6. Tightly cap the bottle of complete growth medium and swirl the contents gently to assure a homogeneous solution. Do not shake forcefully to avoid foaming. Label and date the bottle.

7. Complete growth media should be stored in the dark at 2°C to 8°C (do not freeze). When stored under these conditions, complete growth media is stable for 30 days.

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**Handling Procedure for Frozen Cells and Initiation of Culture**

Refer to the batch specific information for the total number of viable cells recovered from this lot of ATCC® PCS-300-015.

1. Using the total number of viable cells, determine how much surface area can be inoculated to achieve an initial seeding density of between 5,000 and 8,000 cells per cm².

2. Prepare the desired combination of flasks. Add 5 mL of complete growth media per 25 cm² of surface area. Place the flasks in a 37°C, 5% CO₂ humidified incubator and allow the media to pre-equilibrate to temperature and pH for 30 minutes prior to adding cells.

3. While the culture flasks equilibrate, remove one vial of ATCC® PCS-300-015 from storage and thaw the cells by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 1 to 2 minutes).

4. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All operations from this point onward should be carried out under strict aseptic conditions.

5. Add the appropriate volume of complete growth media [volume = (1 mL x number of flasks to be seeded) – 1 mL] into a sterile conical tube. Using a sterile pipette, transfer the cells from the cryovial to the conical tube. Gently pipette the cells to homogenize the suspension. Do not centrifuge.

6. Transfer 1.0 mL of the cell suspension to each of the pre-equilibrated culture flasks prepared in steps 1 to 3 of Handling Procedure for Frozen Cells and Initiation of Culture. Pipette several times, then cap and gently rock each flask to evenly distribute the cells.

7. Place the seeded culture flasks in the incubator at 37°C with a 5% CO₂ atmosphere. Incubate for at least 24 hours before processing the cells further.

**Maintenance**

1. Pre-warm complete growth media (PCS-300-30 and PCS-300-040) in a 37°C water bath. This will take between 10 to 30 minutes, depending on the volume. If using a small volume of medium (50 mL or less), warm only the volume needed in a sterile conical tube. Avoid warming complete growth media multiple times.

2. 24 to 36 hours after seeding, remove the cells from the incubator and view each flask under the microscope to determine percent cellular confluence.

3. Carefully remove the spent media without disturbing the monolayer.

4. Add 5 mL of fresh, pre-warmed complete growth media per 25 cm² of surface area and return the flasks to the incubator.

5. After 24 to 48 hours, view each flask under the microscope to determine percent cellular confluence. If not ready to passage, repeat steps 3 and 4 as described above. When cultures have reached 80% to 90% confluence, and are actively proliferating (many mitotic figures are visible), it is time to subculture. Fibroblasts are not a contact inhibited cell type.

**Subculturing**

1. Passage normal lobar epithelial cells when culture has reached approximately 80% to 90% confluence, and are actively proliferating.

2. Warm both the Trypsin-EDTA for Primary Cells (ATCC® PCS-999-003) and the Trypsin Neutralizing Solution (ATCC® PCS-999-004) to room temperature prior to dissociation. Warm complete growth medium to 37°C prior to use with the cells.

3. For each flask, carefully aspirate the spent media without disturbing the monolayer.

4. Briefly rinse the cell layer with 3 to 5 mL/25 cm² DPBS (ATCC® 30-2200) to remove residual traces of serum and then aspirate and discard the DPBS.

5. Add pre-warmed trypsin-EDTA solution (3-4 mL for every 25 cm²) to each flask.
Primary Lobar Epithelial Cells (ATCC® PCS-300-015™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
1

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Quality Control Specifications

Sterility Testing
Bacteria and yeast: No growth
Mycoplasma: No growth

Viral Testing
Hepatitis B: None detected
Hepatitis C: None detected
Human immunodeficiency virus 1: None detected
Human immunodeficiency virus 2: None detected

Specific Staining
Pan-Cytokeratin (+), TE-7 (-)

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

Human Material Precaution

All tissues used for isolation are obtained under informed consent and conform to HIPAA standards to protect the privacy of the donor’s personal health information. It is best to use caution when handling any human cells. We recommend that all human cells be accorded the same level of biosafety consideration as cells known to carry HIV. With infectious virus assays or viral antigen assays, even a negative test result may leave open the possible existence of a latent viral genome.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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