Bronchial Epithelial Cell Growth Kit (ATCC® PCS-300-040™) contains components that when added to Airway Epithelial Cell Basal Medium (ATCC® PCS-300-030) create a complete ATCC® Primary Cell Solutions™ culture environment for bronchial/tracheal epithelial cells derived from normal human lung (e.g., Primary Bronchial/Tracheal Epithelial Cells, Normal, Human, ATCC® PCS-300-010). The serum-free medium formulation is designed to support normal bronchial/epithelial cell morphology as well as promote rapid growth and proliferation. No feeder layers, extracellular matrix proteins or other substrates are required. Refer to the Certificate of Analysis for batch-specific test results.

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 submersed 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.

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.
3. Obtain one growth kit from the freezer; make sure that the caps of all components are tight.
4. Thaw the components of the growth kit just prior to adding 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.
5. Obtain one bottle of Airway Epithelial Cell Basal Medium (485 mL) from cold storage.
6. Decontaminate the external surfaces of all growth kit component vials and the basal medium bottle by spraying with 70% ethanol.
7. Using aseptic technique and working in a laminar flow hood or biosafety cabinet, transfer the indicated volume of each growth kit component to the bottle of basal medium using a separate sterile pipette for each.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIL Supplement</td>
<td>1.25 mL</td>
<td>HSA 500 µg/mL, Linoleic Acid 0.6 µM, Lecithin 0.6 µg/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 Supplement</td>
<td>5.0 mL</td>
<td>Epinephrine 1.0 µM, Transferrin 5 µg/mL, T3 10 nM, Hydrocortisone 1 µg/mL, rh EGF 5 ng/mL, rh insulin 5 µg/mL</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final Concentration</th>
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Or contact your local distributor

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org
800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

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

**Quality Control Specifications**

**Sterility Testing**
Negative for bacteria, fungi and yeast

**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.

**Disclaimers**

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

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

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