**PneumaCult™-ALI Medium**

Serum- and BPE-free medium for human airway epithelial cells cultured at the air-liquid interface or as airway organoids

Catalog #05001 1 Kit
Catalog #05021 1 Kit
Catalog #05022 1 Kit

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**Product Description**

PneumaCult™-ALI Medium (Catalog #05001) is a serum- and BPE-free medium for the culture of human airway epithelial cells at the air-liquid interface (ALI). Airway epithelial cells cultured in PneumaCult™-ALI Medium undergo extensive mucociliary differentiation to form a pseudostratified epithelium that exhibits morphological and functional characteristics similar to those of the human airway in vivo. PneumaCult™-ALI Medium is also available in a kit that includes 12 mm Transwell® inserts (Catalog #05021) or 6.5 mm Transwell® inserts (Catalog #05022).

Additionally, PneumaCult™-ALI Medium supports the generation of differentiated airway organoids in a 3D culture system. For a detailed protocol, refer to the Technical Bulletin: A Sphere Culture Method for Mucociliary Differentiation of Primary Human Bronchial Epithelial Cells (Document #28216), available at www.stemcell.com or contact us to request a copy.

Together, PneumaCult™-ALI Medium and PneumaCult™-Ex Plus Medium (Catalog #05040) constitute a fully integrated BPE-free culture system for in vitro human airway modeling. This robust and defined system is a valuable tool for basic respiratory research, toxicity studies, and drug development.

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**Ordering Information**

<table>
<thead>
<tr>
<th>PRODUCT NAME</th>
<th>CATALOG #</th>
<th>SIZE</th>
<th>KIT COMPONENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PneumaCult™-ALI Medium</td>
<td>05001</td>
<td>1 Kit</td>
<td>• PneumaCult™-ALI Basal Medium&lt;br&gt;• PneumaCult™-ALI 10X Supplement&lt;br&gt;• PneumaCult™-ALI Maintenance Supplement</td>
</tr>
<tr>
<td>PneumaCult™-ALI Medium with 12 mm Transwell® Inserts</td>
<td>05021</td>
<td>1 Kit</td>
<td>• PneumaCult™-ALI Medium (Catalog #05001)&lt;br&gt;Costar® 12 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts, 48 inserts (Catalog #38023)</td>
</tr>
<tr>
<td>PneumaCult™-ALI Medium with 6.5 mm Transwell® Inserts</td>
<td>05022</td>
<td>1 Kit</td>
<td>• PneumaCult™-ALI Medium (Catalog #05001)&lt;br&gt;Costar® 6.5 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts, 48 inserts (Catalog #38024)</td>
</tr>
</tbody>
</table>

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**Component Storage and Stability**

<table>
<thead>
<tr>
<th>COMPONENT NAME</th>
<th>COMPONENT #</th>
<th>SIZE</th>
<th>STORAGE</th>
<th>SHELF LIFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PneumaCult™-ALI Basal Medium†</td>
<td>05002</td>
<td>450 mL</td>
<td>Store at 2 - 8°C.</td>
<td>Stable for 12 months from date of manufacture (MFG) on label.</td>
</tr>
<tr>
<td>PneumaCult™-ALI 10X Supplement†</td>
<td>05003</td>
<td>50 mL</td>
<td>Store at -20°C.</td>
<td>Stable for 12 months from date of manufacture (MFG) on label.</td>
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<tr>
<td>PneumaCult™-ALI Maintenance Supplement†</td>
<td>05006</td>
<td>5 x 1 mL</td>
<td>Store at -20°C.</td>
<td>Stable for 12 months from date of manufacture (MFG) on label.</td>
</tr>
<tr>
<td>Costar® 12 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts</td>
<td>38023</td>
<td>48 Inserts</td>
<td>Store at 15 - 25°C.</td>
<td>Stable until expiry date (EXP) on label.</td>
</tr>
<tr>
<td>Costar® 6.5 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts</td>
<td>38024</td>
<td>48 Inserts</td>
<td>Store at 15 - 25°C.</td>
<td>Stable until expiry date (EXP) on label.</td>
</tr>
</tbody>
</table>

*This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

†This component is included in the PneumaCult™-ALI Medium kit (Catalog #05001) and is not available for individual sale.
Materials Required But Not Included

<table>
<thead>
<tr>
<th>PRODUCT NAME</th>
<th>CATALOG #</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-PBS (Without Ca++ and Mg++)</td>
<td>37350</td>
</tr>
</tbody>
</table>
| PneumaCult™-Ex Medium  
OR  
PneumaCult™-Ex Plus Medium | 05008  
OR  
05040 |
| Heparin Solution | 07980 |
| Hydrocortisone Stock Solution | 07925 |
| Costar® 12 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts* | 38023*  
OR  
38024** |
| Costar® 6.5 mm Transwell®, 0.4 µm Pore Polyester Membrane Inserts** | 38023*  
OR  
38024** |
| Animal Component-Free Cell Dissociation Kit  
• ACF Enzymatic Dissociation Solution  
• ACF Enzyme Inhibition Solution | 05426 |
| Trypan Blue | 07050 |

*Included in PneumaCult™-ALI Medium with 12 mm Transwell® Inserts kit (Catalog #05021)  
**Included in PneumaCult™-ALI Medium with 6.5 mm Transwell® Inserts kit (Catalog #05022)

Preparation of Reagents and Materials

Use sterile techniques when preparing the following media. If preparing volumes other than the indicated examples, adjust accordingly.

A. PneumaCult™-ALI Complete Base Medium

The following example is for preparing 500 mL of PneumaCult™-ALI Complete Base Medium (PneumaCult™-ALI 10X Supplement + PneumaCult™-ALI Basal Medium). Complete Base Medium is required for preparing Maintenance Medium (section B).

1. Thaw PneumaCult™-ALI 10X Supplement overnight at 2 - 8°C. Mix gently by inverting the vial; do not vortex.  
   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquoted supplement, use immediately. Do not re-freeze.

2. Add 50 mL PneumaCult™-ALI 10X Supplement to 450 mL PneumaCult™-ALI Basal Medium. Mix thoroughly.  
   NOTE: If not used immediately, store PneumaCult™-ALI Complete Base Medium at 2 - 8°C for up to 2 weeks. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the individual components. After thawing Complete Base Medium, use immediately. Do not re-freeze.

B. PneumaCult™-ALI Maintenance Medium

NOTE: Prepare only the volume of PneumaCult™-ALI Maintenance Medium required in section B of Directions for Use (Maintenance Phase).

The following example is for preparing 10 mL of PneumaCult™-ALI Maintenance Medium (PneumaCult™-ALI Complete Base Medium + PneumaCult™-ALI Maintenance Supplement + Heparin Solution + Hydrocortisone Stock Solution).

   NOTE: Once thawed, use immediately or aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquoted supplement, use immediately. Do not re-freeze.

2. Combine the following components:  
   • 9.83 mL PneumaCult™-ALI Complete Base Medium  
   • 100 µL PneumaCult™-ALI Maintenance Supplement  
   • 20 µL Heparin Solution  
   • 50 µL Hydrocortisone Stock Solution  
   NOTE: If not used immediately, store PneumaCult™-ALI Maintenance Medium at 2 - 8°C for up to 2 weeks.
Directions for Use
Please read the entire protocol before proceeding.

A. EXPANSION PHASE (SUBMERGED CULTURE IN INSERTS)
The following example is for passaging human airway epithelial cells from a T-25 cm² flask and plating them onto a single insert (in the 12-well or 24-well format). If using other cultureware, adjust accordingly. PneumaCult™-Ex Medium may be substituted for PneumaCult™-Ex Plus Medium throughout the protocol.

NOTE: For complete instructions on expanding human airway epithelial cells in PneumaCult™-Ex Plus Medium or PneumaCult™-Ex Medium, refer to the Product Information Sheet (Document #DX21576 or 28201, respectively) available at www.stemcell.com or contact us to request a copy.

1. Warm sufficient volumes of D-PBS (Without Ca++ and Mg++), PneumaCult™-Ex Plus Medium, ACF Enzymatic Dissociation Solution, and ACF Inhibition Solution to room temperature (15 - 25°C).
2. Wash cells with 5 mL D-PBS (Without Ca++ and Mg++).
3. Add 2 mL ACF Enzymatic Dissociation Solution and incubate at 37°C for 7 - 8 minutes, until cells can be dislodged with gentle tapping of the flask.
4. Add 2 mL ACF Enzyme Inhibition Solution and collect cells in a 15 mL conical tube (e.g. Catalog #38009).
5. Centrifuge the tube at 350 x g for 5 minutes.
6. Discard the supernatant and resuspend the cell pellet in 1 - 2 mL PneumaCult™-Ex Plus Medium.
7. Perform a viable cell count using Trypan Blue and a hemocytometer.
8. Add PneumaCult™-Ex Plus Medium to one well of the tissue culture plate (basal chamber) as follows:
   • 12-well plate: 1 mL medium
   • 24-well plate: 0.5 mL medium
9. Plate 1 x 10^5 cells/cm² in the insert (apical chamber) as follows:
   • 12 mm Transwell® insert (12-well plate): 11 x 10^4 cells in 0.5 mL PneumaCult™-Ex Plus Medium
   • 6.5 mm Transwell® insert (24-well plate): 3.3 x 10^4 cells in 0.2 mL PneumaCult™-Ex Plus Medium
10. Incubate at 37°C. Perform full medium changes in both the basal and apical chambers every 2 days using PneumaCult™-Ex Plus Medium, until confluence is reached. This typically takes 2 - 4 days.
   NOTE: The expansion phase may take longer for some donor cell populations. Transitioning cultures that are < 50% confluent (PneumaCult™-Ex Plus Medium) or < 80% confluent (PneumaCult™-Ex Medium) is not recommended.
11. Continue to section B (Maintenance Phase).
B. MAINTENANCE PHASE (ALI CULTURE IN INSERTS)

1. Gently aspirate the medium from both the basal and apical chambers. Add PneumaCult™-ALI Maintenance Medium to the basal chamber only, as follows:
   • 12-well plate: 1 mL medium
   • 24-well plate: 0.5 mL medium

2. Incubate at 37°C. Perform a full medium change in the basal chamber using PneumaCult™-ALI Maintenance Medium every 2 days, leaving the apical chamber empty.
   NOTE: On weekends, change the medium on Friday afternoon and Monday morning.

3. Beginning in week 2 post-airlift, remove excess mucus from the apical surface by washing the cells once with D-PBS (Without Ca++ and Mg++) at room temperature (15 - 25°C) as follows:
   • 12-well plate: 0.5 mL D-PBS
   • 24-well plate: 0.2 mL D-PBS
   This procedure should be repeated as required (approximately once per week) to prevent excessive mucus accumulation.
   NOTE: Take care when removing liquid to avoid damaging the underlying cells.

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