Primary Lung Fibroblasts; Cystic Fibrosis (ATCC® PCS-201-016™)

Described Cell Characteristics

Tissue: respiratory tract

Morphology: Spindle-shaped; cells are bipolar and refractile.

Growth Properties: adherent

Preparation of Complete Growth Medium

1. Obtain one vial of Primary Lung fibroblast cells ; Normal, Human (ATCC PCS-201-013) from the liquid nitrogen tank; make sure that the caps of all components are tight.
2. Thaw the components of Fibroblast Growth Kit-Low serum ATCC® PCS-201-041™ just prior to adding them to the Fibroblast Basal Medium ATCC® PCS-201-030™.
3. Obtain one bottle of Fibroblast Basal Medium ATCC® PCS-201-030™ 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, into the bottle of basal medium using a separate sterile pipette for each transfer.

Table 1. Fibroblast Growth Kit-Low Serum Components

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>rh FGF b</td>
<td>0.5 mL</td>
<td>5 ng/mL</td>
</tr>
<tr>
<td>L-glutamine</td>
<td>18.75 mL</td>
<td>7.5 mM</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.5 mL</td>
<td>50 µg/mL</td>
</tr>
<tr>
<td>Hydrocortisone Hemisuccinate</td>
<td>0.5 mL</td>
<td>1 µg/mL</td>
</tr>
<tr>
<td>rh Insulin</td>
<td>0.5 mL</td>
<td>5 µg/mL</td>
</tr>
<tr>
<td>Fetal Bovine Serum</td>
<td>10.0 mL</td>
<td>2%</td>
</tr>
</tbody>
</table>

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.

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 cells.

1. Using the total number of viable cells, determine how much surface area can be inoculated to achieve an initial seeding density of between 2,500 and 5,000 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
Please read this FIRST

Storage Temp. 
liquid nitrogen vapor phase

Biosafety Level
1

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 Lung Fibroblasts; Cystic Fibrosis (ATCC® PCS-201-016™)

Maintenance
1. Pre-warm complete growth media 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. To 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 lung fibroblast 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 DPBS (ATCC 30-2200) to remove residual traces of serum and then aspirate and discard the DPBS.
5. Add pre-warmed trypsin-EDTA solution (2 to 3 mL for every 25 cm2) to each flask.
6. Gently rock each flask to ensure complete coverage of the trypsin-EDTA solution over the cells.
7. Observe the cells under the microscope. When the majority of the cells are detached, quickly add an equal volume of Trypsin Neutralizing Solution (ATCC PCS-999-004) to each flask. Gently pipette or swirl the culture to ensure all of the trypsin-EDTA solution has been neutralized.
8. When the majority of cells are detached, quickly add an equal volume of Trypsin Neutralizing Solution (ATCC PCS-999-004) to each flask. Gently pipette or swirl the culture to ensure all of the trypsin-EDTA solution has been neutralized.
9. Transfer the dissociated cells to a sterile centrifuge tube and set aside while processing any remaining cells in the flask.
10. Add 3 to 5 mL Trypsin Neutralizing Solution (ATCC PCS-999-004) to the flask to collect any remaining dissociated cells. Transfer remaining cells into the centrifuge tube.
11. Repeat steps 10 as needed until all cells have been collected from the flask.
12. Centrifuge the cells at 150 x g for 3 to 5 minutes.
13. Carefully aspirate the neutralized dissociation solution from the cell pellet and re-suspend the cells in 5 to 8 mL fresh, pre-warmed, complete growth medium.
14. Count the cells and seed new flasks at a density of 2,500 to 5,000 cells per cm2.
15. Place freshly seeded flasks in a 37°C, 5% CO2 incubator for at least 24 to 48 hours before processing the cells further. Refer to Maintenance for guidelines on feeding.

Viability: Refer to Certificate of Analysis for lot specific data

Quality Control Specifications
Sterility Testing
Bacteria and Yeast: Negative
Mycoplasma: Negative

Viral Testing
Hepatitis B: Negative
Hepatitis C: Negative
HIV: Negative

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

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.

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

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