Thawing and Planting Protocol:

Human Lung Cancer Associated Fibroblasts (LCAF) are fibroblast cells isolated from human primary non-small cell lung cancer tumors. LCAF can be successfully cultured for multiple passages although the upper pass number is not known. The following is the recommended protocol for thawing and subculturing of these cells.

Note: Once complete media has been formulated, it should be stored at 4°C. Avoid extended exposure of the medium to room or higher temperatures. Medium should be equilibrated in a water bath set at 37°C before adding media to any cell culture.

**Thawing Cells**

1. Remove the vial of cells from dry ice. Defrost the vial of cells in a 37°C water bath until ice in the ampoule is no longer visible.
2. Immediately disinfect the vial with 70% isopropanol.
3. Working in a laminar flow hood, open the vial and transfer the contents to a sterile 15 mL tube.
4. Very slowly, add approximately 10 mL of complete Lung Cancer Associated Fibroblast Expansion Media (Table 1) pre-warmed to 37°C.
5. Centrifuge the suspended cells at 200 x g for 10 minutes.
6. Decant the medium and gently re-suspend the pellet in 10 mL of complete Lung Cancer Associated Fibroblast Expansion Media (Table 1), then transfer into a T-75 (75 cm²) culture flask.
7. Observe the cells microscopically to estimate cell viability and place the flask in an incubator at 37°C, 5% CO2 and 90% humidity.
8. Cells will be ready to pass between 8 to 10 days. Cells should be subcultured at a density of 10,000 to 20,000 cells/cm² or desired plating density.

**Table 1. Preparation of 500 mL complete Lung Cancer Associated Fibroblast Expansion Media**

<table>
<thead>
<tr>
<th>Brand</th>
<th>Amount for 500 mL</th>
<th>Product</th>
<th>Catalog #</th>
</tr>
</thead>
<tbody>
<tr>
<td>CET</td>
<td>450 mL</td>
<td>CET Lung Cancer Associated Fibroblast Expansion Media</td>
<td>HLCAFE.Media-450</td>
</tr>
<tr>
<td>Any</td>
<td>50 mL</td>
<td>Fetal Bovine Serum</td>
<td>Refer to Manufacture’s Catalog Number</td>
</tr>
</tbody>
</table>

Store media at 4°C

**Key References:**


Certificate of Analysis

All hematopoietic, mesenchymal and multipotent stem cells are evaluated by flow cytometry for specific stem cell markers. All other cells are evaluated either by staining, method of isolation or traditional molecular biology techniques. Data is available upon request.

All growth and differentiation media are evaluated by conducting assays to make sure cells either grow or differentiate as indicated on the media label. Data is available upon request.

All cells are tested for HIV-1, HIV-2, Hepatitis B and Hepatitis C using sensitive PCR based assays. All cells test negative for these viruses. However, all human cells must be used in accordance with established laboratory safety procedures and only under the supervision of trained personnel.

All products are for research use only. Not for diagnostic or therapeutic use. CET's products are designed and tested to function with other CET products only. For example, all of our cells are optimized to grow and differentiate in CET media. Although investigators are welcome to formulate their own media, CET cannot and will not guarantee that cells will function as indicated in the product brochure. Moreover, such third party use will void CET's obligation to replace cells, should they not function as indicated.