Lung Cancer Associated Fibroblasts

HNSC.CLAF-500 Store cells at -80ºC or in liquid nitrogen

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.

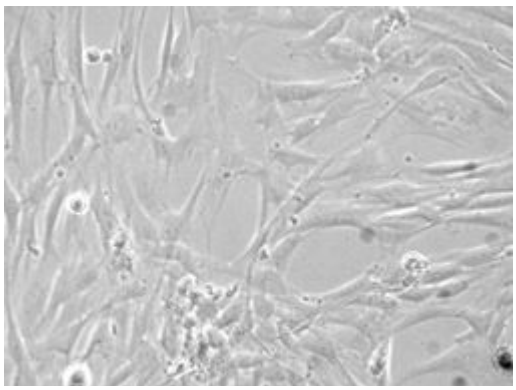

Figure 1: Lung Cancer Associated Fibroblasts

Note: Antibiotics/ antimycotics should not be used as an alternative to proper aseptic technique.

Key References:

1. Highly Variable Response to Cytotoxic Chemotherapy in Carcinoma Associated Fibroblasts (CAFs) From Lung and Breast. Sonnenberg M, van der Kuip H, Haubeisz S, Fritz P, Schroth W, Friedel G, Simon W, Murdter TE, Aulitzky WE. BMC Cancer. 2008 Dec 11;8(1):364.

2. Proliferative Stimulus of Lung Fibroblasts on Lung Cancer Cells Is Impaired by the Receptor for Advanced Glycation End-Products. Bartling B, Demling N, Silber RE, Simm A. Am. J.Resp. Cell Mol. Bio. 2006. 34:83-91.

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

Brand	Amount for 500 mL	Product	Catalog #
CET	450 mL	CET Lung Cancer Associated Fibroblast Expansion Media	HLCAF.E.Media-450
Any	50 mL	Fetal Bovine Serum	Refer to Manufacture's Catalog Number

Store media at 4ºC

Cellular Engineering Technologies, Inc.
2500 Crosspark Rd., E110, Coralville IA 52241
Phone: 319-665-3000 Fax: 319-665-3003

CET
celleng-tech.com

Certificate of Analysis

All hematopoietic, mesenchymal and multipotent stem cells are evaluated by flow cytometry for specific stem cell mark-ers. 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 nega-tive 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.

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