



## Immortalized Human Lung Squamous Cell Carcinoma CAFs

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**Catalog #:** CAF117S-IM

**Cell #:** >1x10<sup>6</sup> cells

**Storage:** Liquid Nitrogen until ready for culture.  
While Culturing keep in 37°C CO<sub>2</sub> incubator

**Product Format:** Frozen Vial

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### GENERAL INFORMATION

Carcinoma associated fibroblasts (CAFs) have recently been implicated in important aspects of epithelial solid tumor biology such as neoplastic progression, tumor growth, angiogenesis, and metastasis. CAFs isolated from lung squamous cell carcinoma tissue were infected with hTert-lentiviral particles under specially formulated media. A Puromycin resistant gene was expressed under RSV promoter, which allows selecting the transduced cell via Puromycin antibiotic hTERT. Normal CAFs can be passaged 3-5 times, while immortalized CAFs can be passaged >10 times.

*Product is for Research use only.*

Frozen Vials are shipped in a Dry Ice Package.

### PRODUCT TESTING

- Negative for bacteria, yeast, fungi, and mycoplasma
- Expression of CAF markers, including smooth muscle actin alpha (actin alpha), PDGF receptor alpha (CD140a), PDGF receptor beta (CD140b), fibroblast specific protein 1(FSP-1/S100A4) and fibroblast activation protein (FAP)

### HANDLING OF ARRIVING CELLS

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. To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

### MEDIUM

We recommend customers use our CAF Growth Media (cat. CAFM03) to culture these cells.

### FOR RESEARCH USE ONLY

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## PROTOCOL FOR THAWING THE CELLS

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed. Decontaminate by dipping in or spraying with 70% ethanol. All the operations from this point on should be carried out under strict aseptic conditions.
3. It is recommended that the cryoprotective agent be removed immediately. Centrifuge the cell suspension at approximately 125 x g for 5 to 10 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium.
4. Add 6.0 to 8.0 mL of AlphaBioCoat (cat. AC001) to the T-Flask for 15 minutes. Aspirate the solution after 15 minutes, rinse with 8ml of 1XPBS. Discard the 1XPBS. Transfer the cells to an appropriate size T-Flask.
5. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).
6. Incubate the culture at 37°C in a suitable incubator. A 5% CO<sub>2</sub> in air atmosphere is recommended if using the medium described on this product sheet.

## SUBCULTURING PROCEDURE

**Note:** If you have any questions or need clarification regarding the protocol for culturing these cells, please reach out to Dr. Jensen Auguste at (978) 608-1766 with your questions before beginning.

**Note:** Volumes are given for a 75 cm<sup>2</sup> flask. Increase or decrease the amount of medium needed proportionally for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 1x PBS, remove and discard 1x PBS.
3. Add 2.0 to 3.0 mL of Cell Detachment solution (cat. ADF001) to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
  - **Note:** To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of AlphaBioCoat to the T-Flask for 15 minutes. Aspirate the solution after 15 minutes, rinse with 8ml of 1XPBS. Discard the 1XPBS.
5. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
6. To remove cell detachment solution, transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes. Discard supernatant and resuspend cells in fresh serum-free growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
7. Place culture vessels in incubators at 37°C.

**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:3 is recommended

**Medium Renewal:** Every 3 to 4 days

**Reagent for cryopreservation:** Complete growth medium supplemented with 5% DMSO

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