



## Human Lung Fibroblast Cells

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**Catalog #:** HLF001

**Cell #:** >5x10<sup>5</sup> cells

**Storage:** Liquid Nitrogen until ready for culture.  
While culturing keep in 37°C CO<sub>2</sub> incubator (95% air, 5% CO<sub>2</sub>)

**Product Format:** Frozen Vial

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### GENERAL INFORMATION

Human cells exhibiting fibroblast morphology isolated from normal lung tissue. Cell populations are offered at passage 1 in a frozen vial. It is recommended to culture these cells following the protocols described below.

*Product is for Research use only.*

Frozen Vials are shipped in a Dry Ice Package.

### STATEMENT

Handling human tissue derived products is potentially bio-hazardous, despite testing negative for HIV, HBV, and HCV DNA. Nonetheless, proper precautions must be taken to avoid inadvertent exposure.

### SPECIAL NOTES:

- We strongly advise our customers to use medium and related products recommended by Neuromics, because our cells were grown and adapted using our products.
- The growth featured of our cells cannot be guaranteed if the specific growth mediums stated in our datasheets are not used.
- Due to the sensitive nature of primary cells and cell lines, all quality related issues about the cells must be reported back to us within one month after receiving the products. Cells will not be replaced after the one-month period.

### UNPACKING AND STORAGE INSTRUCTIONS

1. Check all containers for leakage or breakage. 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.
2. Complete medium: The base medium for this cell line is Lung Fibroblast Cell Media (CCM005). To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

### HANDLING PROCEDURE

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. 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).

### FOR RESEARCH USE ONLY

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1. Remove the vial from the water bath as soon as the contents are thawed and decontaminate by dipping in or spraying with 70% ethanol. All the operations from this point on should be carried out under strict aseptic conditions.
2. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium (see the specific batch information for the culture recommended dilution ratio) and spin at approximately 125 x g for 5 to 10 minutes.
3. Resuspend cell pellet with the recommended complete medium. and dispense into a 25 cm<sup>2</sup> or a 75 cm<sup>2</sup> culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells.
4. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete 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).
5. 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.

## PROTOCOL FOR THAWING THE CELLS AND SUBCULTURE

**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 used in this protocol are for 75 cm<sup>2</sup> flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium. Briefly rinse the cell layer with 1XPBS to remove all traces of serum that contains inhibitor.
2. Add 2.0 to 3.0 mL of Cell Detachment solution (ADF001) to the flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
  - a. **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.
3. Add 6.0 to 8.0 mL of AlphaBioCoat (AC001) to the T-Flask for 15 minutes. Aspirate the solution after 15 minutes, rinse with 8ml of 1XPBS. Discard the 1XPBS.
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:8 is recommended.

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

**Note:** Growth of the cells is enhanced by the addition of tumor necrosis factor-alpha (TNF alpha) to the medium.  
Reagents for cryopreservation: Complete growth medium supplemented with 5% (v/v) DMSO.

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