EML4-ALK Fusion-A549 Isogenic Cell Line Human (ATCC® CCL-185IG™)

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.

Complete Growth Medium
The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Citation of Strain
If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: EML4-ALK Fusion-A549 Isogenic Cell Line Human (ATCC® CCL-185IG™)

Citation of Strain

Organism: Homo sapiens, human
Tissue: lung
Disease: Carcinoma
Cell Type: epithelial
Age: 58 years
Gender: male
Morphology: epithelial-like
Growth Properties: adherent
Isoenzymes: G6PD, B
DNA Profile: Amelogenin: X
CSF1PO: 10,12
D13S317: 11
D16S539: 11,12
D5S818: 11
D7S820: 8,11
TH01: 8,9,3
TPOX: 8,11
vWA: 14

Unpacking & Storage Instructions
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.

Handling Procedure for Frozen Cells
To insure 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, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium, and spin at approximately 125 x g for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). 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 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). pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures
The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination.
If the cells are still attached, aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.

3. If the cells are not attached, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.

**Subculturing Procedure**

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Conical T-75 flasks (catalog #306461) are recommended for subculturing this product.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53 mM EDTA Solution (ATCC® 30-2101) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution 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 complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
   - Cultures can be established between 2 x 10³ and 1 x 10⁴ viable cells/cm². Do not exceed 7 x 10⁴ cells/cm².
6. Incubate cultures at 37°C.

**Subcultivation Ratio**: A subcultivation ratio of 1:3 to 1:5 is recommended

**Medium Renewal**: 2 to 3 times per week

**Comments**

The anaplastic lymphoma kinase (ALK) genetic abnormality is a key oncogenic driver, especially in non-small cell lung cancer. The EML4-ALK gene fusion caused by a chromosomal inversion can produce constitutively active ALK tyrosine kinase protein, which leads to enhanced cell survival and cell proliferation. There are multiple EML4-ALK fusion variants, with the most prevalent being variant 1 (E13; A20) in which EML4 intron 13 is fused with ALK intron 20. ATCC EML4-ALK fusion variant 1 (E13; A20). This isogenic cell line (CCL-185IG) was created from the A549 parental cell line, CCL-185, a non-small cell lung cancer cell line. The EML4-ALK fusion in CCL-185IG has been extensively validated at the genomic, transcript and protein levels. Furthermore, CCL-185IG is more sensitive to the ALK inhibitor crizotinib when compared to its parental cell line. CCL-185IG can be a useful model to study tyrosine kinase signaling pathway, and to screen ALK inhibitors in anti-cancer drug discovery and development.

**References**

References and other information relating to this product are available online at www.atcc.org.

**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.

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

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