Adherent

This is a near-diploid human cell line of female origin with a modal chromosome count of 46 and a polyploidy rate of 27%. There were two copies of a karyotypically normal X-chromosome present in most of the cells. Overall, some of the cells contained chromosomal abnormalities, with most consistent being trisomy 20.

Complete Growth Medium

These cells are grown in a serum-free medium: BEGM (Bronchial Epithelial Growth Medium, Serum-free) from Lonza (BEGM Bullet Kit; CC-3170) made of BEBM basal medium and SingleQuot additives (ATCC does not use gentamycin-amphotericin B) supplemented with 50 µg/ml G-418.

Cytogenetic Analysis: This is a near-diploid human cell line of female origin with a modal chromosome count of 46 and a polyploidy rate of 27%. There were two copies of a karyotypically normal X-chromosome present in most of the cells. Overall, some of the cells contained chromosomal abnormalities, with most consistent being trisomy 20.

DNA Profile:
Amelogenin: X
CSF1PO: 12
D13S317: 11,13
D16S539: 11,14
D5S818: 12
D7S820: 8,11
THO1: 6,9,3
TPOX: 8,11
vWA: 17,20

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: CuFi-1 (ATCC® CRL-4013™)

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

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Handling Procedure for Flask Cultures

The flask was seeded with cells, incubated, 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. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached**, aseptically remove all but 5 to 10 mL of the shipping medium. 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 all but 5 to 10 mL of the shipping medium. Resuspend the pellet cells in the 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.

Population Doubling Capacity

As part of our quality control, we have tested this cell line for its ability to grow for a minimum of 15 population doublings after recovery from cryopreservation. We have also compared its karyotype, telomerase expression level, growth rate, morphology and tissue-specific markers when first recovered from cryopreservation with that of cells at 10+ population doublings to ensure that there is no change in these parameters and that the cells are capable of extended proliferation.

Subculturing Procedure

**Note:** The culture flasks should be pre-coated with 60 μg/mL solution of Human Placental Collagen Type IV. (Sigma Cat. No. C-7521) at least 18 hours in advance then air-dried and rinsed 2-3 times with Dulbecco's Phosphate Buffered Saline.

Volumes used in this protocol are for 75 cm² flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. 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.
3. To remove Trypsin-EDTA solution, add 2.0 to 3.0 mL of 1% FBS in Dulbecco's Phosphate Buffered Saline and aspirate cells by gently pipetting.
4. Transfer cell suspension to a centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes. Discard supernatant.
5. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 1 x 10⁶ to 3 x 10⁷ viable cells/cm² is recommended.
6. Incubate cultures at 37°C.
7. Subculture when cell concentration is between 1 x 10⁶ and 2 x 10⁷ cells/cm².

Subcultivation Ratio: 1:5

Medium Renewal: Every 2-3 days (do not exceed 3 days)


Cryopreservation Medium

BEGM supplemented with 10% (v/v) DMSO and 30% (v/v) fetal bovine serum.

Store in liquid nitrogen vapor. Avoid immersing vials into liquid nitrogen.

Comments

The cells do not undergo growth arrest in cell culture due to exogenous expression of the telomerase and E6/E7 genes. CuFi-1 cells are homozygous for the delta F508 cystic fibrosis-causing mutation (delta F508/delta F508).

Another hTERT-immortalized cell line, derived from normal HAE is also available as ATCC CRL-4011 (NuLi-1). Both cell lines, when seeded on semipermeable filters and grown at the air-liquid interface, are capable of forming polarized differentiated epithelia that exhibit transepithelial resistance and maintain the ion channel physiology expected of each genotype.
References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 2

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.

ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

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