





Product Sheet

NuLi-1 (ATCC® CRL-4011™)

Please read this FIRST



Storage Temp.
**Liquid nitrogen
vapor phase**



Biosafety Level
2

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

These cells are grown in a serum-free medium: Airway Epithelial Cell Basal Medium (ATCC PCS-300-030) and Bronchial Epithelial Cell Growth Kit (ATCC PCS-300-040) additives. Atmosphere: air, 95%; CO₂, 5%

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: NuLi-1 (ATCC® CRL-4011™)

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Description

Organism: *Homo sapiens*, human
Immortalization Method: HPV-16 E6/E7 and hTERT expression
Tissue: Lung; epithelium, bronchus
Disease: Normal
Cell Type: Epithelial cells immortalized with E6/E7 and hTERT
Age: 36 years
Gender: Male
Morphology: Epithelial-like
Growth Properties: Adherent
DNA Profile:
CSF1PO: 11
D13S317: 10,12
D16S539: 8,13
D5S818: 11,13
D7S820: 12,13
TH01: 9.3
TPOX: 8,11
vWA: 14,18
Amelogenin: X,Y

Cytogenetic Analysis: This is a near-diploid human cell line of male origin with a polyploidy rate of 24%. There were copies of karyotypically normal X and Y-chromosomes present in most of the cells analyzed. Overall, some of the cells contained chromosomal abnormalities, with the most consistent being trisomy 5 and 20.

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

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


1. Prepare a 25 cm² or a 75 cm² culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents, the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.
2. 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).
3. 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 operations from this point on should be carried out under strict aseptic conditions.
4. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge the cell suspension at approximately 125 x g for 5 to 7 minutes.
5. Discard the supernatant and resuspend the cells in fresh growth medium (see the batch-specific information for the recommended dilution ratio). Add this suspension to the prepared culture vessel.
6. Incubate the culture at 37°C in a suitable incubator.
7. A 5% CO₂/95% air atmosphere is recommended if using the medium described on this product sheet.




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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 pelleted 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 or equivalent) at least 18 hours in advance then air-dried and rinsed 2-3 times with Dulbecco's Phosphate Buffered Saline. Volumes are given for a 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Subculture when the culture is about 90% confluence. Expected cell yield is between 4 x 10⁴ and 5 x 10⁴ viable cells/cm².
2. Remove and discard culture medium.
3. Add 2.0 to 3.0 mL of 0.25% (w/v) trypsin-0.53 mM EDTA solution to the flask and observe cells under an inverted microscope until the cell layer is dispersed (usually within 5 to 10 minutes). **Note:** To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Place at 37°C to facilitate dispersal.
4. To stop trypsinization, add 2.0 to 3.0 mL of 1% FBS in Dulbecco's Phosphate Buffered Saline and aspirate cells by gently pipetting.
5. Transfer cell suspension to a 15 mL centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes.
6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 1.5 to 2.0 x 10⁴ viable cells/cm² is recommended.
7. Incubate cultures at 37°C.

Subcultivation Ratio: 1:3

Medium Renewal: Every 2-3 days (**DO NOT EXCEED 3 DAYS**)

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in Culture Of Animal Cells: A Manual of Basic Techniques by R. Ian Freshney, 5th edition, published by Wiley-Liss, N.Y., 2005.



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 HPV-16 E6/E7 genes.

Another hTERT-immortalized cell line, derived from HAE of a cystic fibrosis patient is available as ATCC® CRL-4013™ (CuFi-1). Both cell lines, when seeded on semi-permeable 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.

At this time, ATCC does not include functionality tests like those outlined in Zabner et al [Pubmed: 12676769].

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References

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.

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

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