





Product Sheet

hTERT NF1 ipnNF95.11c (ATCC® CRL-3391™)

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

The basal medium for this cell line is Dulbecco's Modified Eagle's Medium (DMEM; ATCC 30-2002). To make the complete medium add the following components to 500 mL DMEM:

- 56 mL FBS (ATCC 30-2020)
- 5.6 mL L-glutamine from stock 200mM (ATCC 30-2214)

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: hTERT NF1 ipnNF95.11c (ATCC® CRL-3391™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Homo sapiens*, human

Tissue: Unaffected (non-tumor) peripheral nerve from NF1 patient. Primary site: peripheral nerve in trunk.

Disease: plexiform neurofibroma; tumor not malignant

Cell Type: Schwann cell

Age: 32 years old

Gender: male

Morphology: fibroblast/spindle-like

Growth Properties: adherent

DNA Profile:

Amelogenin: X,Y

CSF1PO: 10,11

D13S317: 11

D16S539: 13,14

D5S818: 11

D7S820: 10,12

THO1: 8,9

TPOX: 8,11

vWA: 16

Cytogenetic Analysis: 46,XY (p24)

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 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.




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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. 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. Corning® T-75 flasks (catalog #430641) 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 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 1.5 x 10⁴ and 3.0 x 10⁴ viable cells/cm².
6. Incubate cultures at 37°C.

Interval: Maintain cultures at a cell concentration between 8.0 X 10³ and 1.9 X 10⁵ cell/cm².

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended

Medium Renewal: 2 to 3 times per week



Cryopreservation Medium

Culture media + 10% DMSO (ATCC 4-X)



Comments

A team of researchers from the University of Florida, the National Center for Advancing Translational Sciences (NCATS), and Sage Bionetworks with support from the Neurofibromatosis Therapeutic Acceleration Program (NTAP) at Johns Hopkins, have developed and characterized a set of immortalized patient derived plexiform neurofibroma Schwann cells in an initial effort to create a series of cells that represent the genetic diversity of human plexiform tumors.¹

Through generous support from the Neurofibromatosis Therapeutic Acceleration Program (NTAP), purchasers of these cells are eligible to receive up to a 100% reimbursement from NTAP. For additional information and to apply for reimbursement after a purchase, please contact NTAP at info@n-tap.org or visit the NTAP Website.

Through exogenous expression of human telomerase reverse transcriptase (hTERT) and murine cyclin-dependent kinase (mCdk4) using retroviral (and subsequently lentiviral) vectors carrying the hTERT and mCdk4 genes, normal (NF1 wild-type, +/+), neurofibroma-derived Schwann cells that were heterozygous (+/-) for NF1 mutation, and homozygous (-/-) for NF1 mutation, were immortalized.¹

This product, and other immortalized cells from the collection, have undergone in-depth characterization and authentication.

Real time PCR for hTERT and mCdk4	Proliferation rate
NF1 mutational analysis	Doubling time
Karyotyping	Apoptosis
Cell authentication	Microsatellite instability
Contact inhibition	Tumorigenicity

To learn more and view characterization data, go to:

<https://www.synapse.org/#!Synapse:syn7239479/wiki/405899>

Gosline, SJC Synapse <http://doi.org/10.7303/syn4939906> (2017).



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Gosline, SJC Synapse <http://doi.org/10.7303/syn4940963> (2017).

Gosline, SJC Synapse <http://doi.org/10.7303/syn4939874> (2017).

NCBI Sequence Read Archive [SRP125359](https://www.ncbi.nlm.nih.gov/sra/SRP125359) (2018)

- Mixture of 6 single-cell clones derived from P16 immortalized primary culture of plexiform neurofibroma cells (ipNF05.5)
- Tumor: homozygous disease (NF1-/-), plexiform neurofibroma
- Oncogenes: transduced with mCdk4 cDNA and hTERT
- Neurofibroma Schwann cell culture transduced with human telomerase (TERT) cDNA and mouse Cdk4 cDNA in lentivirus vectors (no selection), to immortalize the cells.

Proliferation rate: 82% BrdU positive at 75% confluency



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.

ATCC Warranty

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Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans. While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

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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.

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