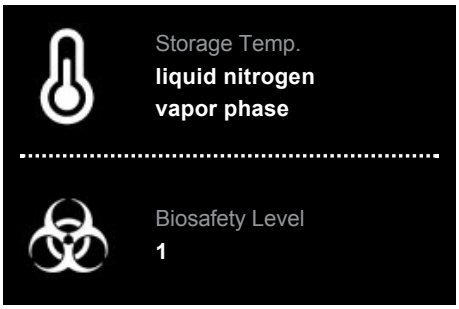
ATCC

Product Sheet

NCI-H446 [H446] (ATCC® HTB-171™)

Please read this FIRST



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 RPMI-1640 Medium, ATCC 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum (ATCC 30-2020) 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: NCI-H446 [H446] (ATCC® HTB-171™)

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: lung; derived from metastatic site: pleural effusion
Disease: carcinoma; small cell lung cancer
Age: 61 years
Gender: male
Morphology: epithelial
Growth Properties: mixed, adherent and suspension
Isoenzymes:
AK-1, 1
ES-D, 1
G6PD, B
GLO-I, 2
Me-2, 0
PGM1, 1-2
PGM3, 1
DNA Profile:
Amelogenin: X,Y
CSF1PO: 13,14
D13S317: 8
D16S539: 12
D5S818: 11
D7S820: 10,11
THO1: 8,9.3
TPOX: 9,11
vWA: 18,19
Cytogenetic Analysis: This is a hypertriploid human cell line. The modal chromosome number is 74, occurring at 22% with polyploidy at 2.5%. Over 25 markers were common to most cells including inv(1)(p32q21), der(1)t(1:4)(q42;q11), i(5q), t(2p13q) and der(19)t(7;HSR;19) (q11;HSR;p13.3). Structurally normal N1 and N13 were absent and normal N4, N5 and N10 were mostly single copies per cell. There were two X/Y pairs per cell.

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 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 an appropriate size vessel. 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 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).

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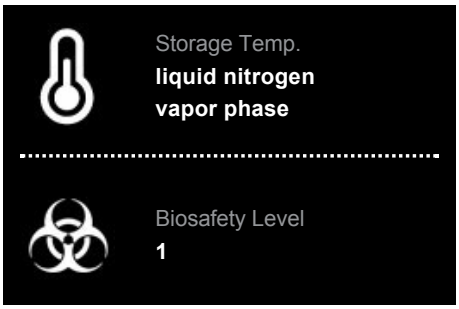

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4. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

If it is desired that the cryoprotective agent be removed immediately, or that a more concentrated cell suspension be obtained, centrifuge the cell suspension at approximately 125 x g for 5 to 10 minutes. Discard the supernatant and resuspend the cells with fresh growth medium at the dilution ratio recommended in the specific batch information.

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. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination
2. Incubate the flask in an upright position for several hours at 37°C. After the temperature has equilibrated, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save for reuse. Resuspend the cell pellet in 10 mL of this medium.
3. From this cell suspension remove a sample for a cell count and viability.
4. Incubate the culture, horizontally, at 37°C in a 5% CO_2 in air atmosphere.

Subculturing Procedure

This is a cell line that grows as both attached and suspended cells. The suspended cells are viable and can be used for subculture.To subculture the attached cells, remove the old medium (recover the suspended cells by centrifugation if desired), rinse the monolayer with fresh 0.25% trypsin, 0.53 mM EDTA and let the culture sit at 37°C until the cells detach. Add fresh medium, disperse cells and transfer to a new flask.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:9 is recommended
Medium Renewal: 2 to 3 times per week

Cryopreservation Medium

Complete culture medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Comments

The morphology of the original tumor was not characteristic of SCLC.
The line is a biochemical and morphological variant of SCLC that expresses neuron specific enolase and the brain isoenzyme of creatine kinase.
It does not have detectable levels of L-DOPA decarboxylase, bombesin, vasopressin, oxytocin or gastrin releasing peptide.
C-myc DNA sequences are amplified about 20 fold, and there is a 15 fold increase in c-myc RNA relative to normal cells.
The line was originally propagated in serum free RPMI 1640 medium supplemented with 10 nM hydrocortisone, 0.005 mg/mL insulin, 0.01 mg/mL transferrin, 10 nM 17-beta-estradiol, and 30 nM sodium selenite.
The cells form transplantable tumors with non-typical SCLC histology.

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.

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

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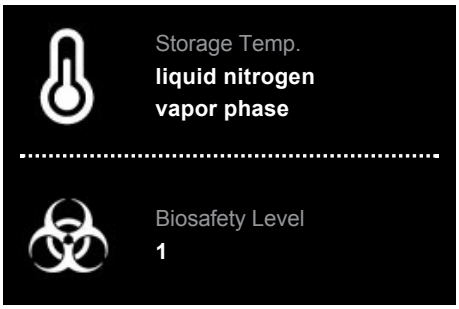

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media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

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.
This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.
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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