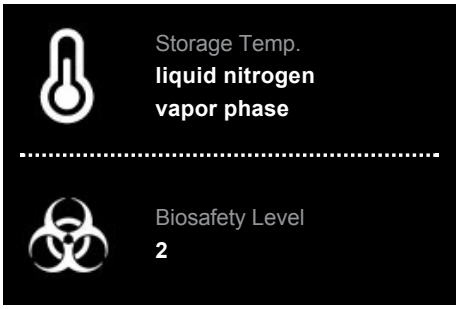
ATCC

Product Sheet

HBE4-E6/E7 [NBE4-E6/E7] (ATCC® CRL-2078™)

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 provided by Invitrogen (GIBCO) as part of a kit: Keratinocyte Serum Free Medium (K-SFM), Kit Catalog Number 17005-042. This kit is supplied with two additives required to grow this cell line (bovine pituitary extract (BPE) and human recombinant epidermal growth factor (EGF). To make the complete growth medium, you will need to add the following components to the base medium:

- 0.05 mg/ml BPE - provided with the K-SFM kit
- 5 ng/ml EGF - provided with the K-SFM kit
- 10 ng/ml cholera toxin - not provided with kit

NOTE: Do not filter complete medium

Citation of Strain

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American Type Culture Collection
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Description

Organism: *Homo sapiens*, human
Tissue: lung; bronchus
Disease: Adenocarcinoma, Papilloma
Cell Type: epithelial; human papillomavirus 16 (HPV-16) E6/E7 transformed
Age: 60 years
Gender: male
Morphology: epithelial
Growth Properties: adherent
DNA Profile:
Amelogenin: X,Y
CSF1PO: 11,12
D13S317: 11
D16S539: 11,13
D5S818: 10,11
D7S820: 10,11
THO1: 6,8
TPOX: 8
vWA: 14,17
Cytogenetic Analysis: 45, X, -Y, dup (5), -8, +9, -20, -22, +mar1, +mar2

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 a 15 ml centrifuge tube and dilute with the recommended complete culture medium.
4. Centrifuge the cell suspension at approximately 125 xg for 5 to 10 minutes. Discard the supernatant and resuspend the cells with fresh medium at the dilution ratio recommended in the specific batch information.. 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).
5. 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.

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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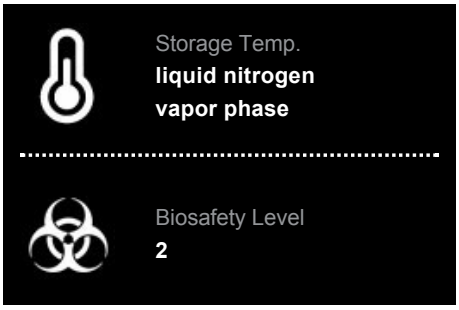

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1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check 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% CO_2 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 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution containing 0.5% polyvinylpyrrolidone (PVP).
3. Add 2.0 to 3.0 mL of Trypsin-EDTA-PVP 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 2.0 to 3.0 mL of complete growth medium containing 0.1% soybean trypsin inhibitor and 0.1% bovine serum albumin and aspirate cells by gently pipetting.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.
6. Incubate cultures at 37°C.

Subculture before the cells become confluent.
Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:4 is recommended
Medium Renewal: Every 2 to 3 days

Cryopreservation Medium

Growth medium with 10% glycerol.

Comments

These sequences are known to bind to and inactivate endogenous p53 and Rb proteins respectively.
Southern blot analysis shows that stable integration of the transfected genes occurred.
The cell line resembles morphologically the basal cells of the normal human bronchial epithelium, and is not tumorigenic in athymic nude mice.
The cells are able to form tubules when grown in a basement membrane like matrix, and they retain the ability to undergo terminal squamous differentiation in response to phorbol esters or upon reaching confluence.
Cells are non-viable in DMSO and should be frozen in culture Medium with 10% glycerol.
Original authentication testing at ATCC by isoenzymology indicated that this was a human cell line. Recent additional speciation using a mitochondrial cytochrome c oxidase I (CO1) assay has shown that this cell line is cross-contaminated with bovine cells. Additional more sensitive PCR assays of the original token lot have also indicated a low level of bovine cross-contamination in that material as well.

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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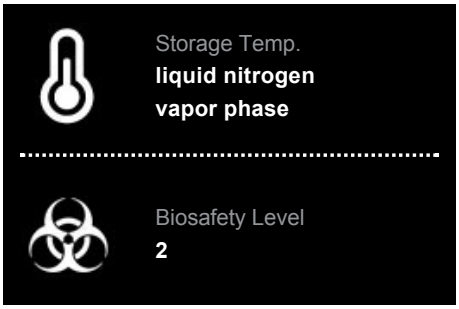

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Disclaimers

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.