ATCC

Product Sheet

BEAS-2B (ATCC® CRL-9609™)

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 base medium for this cell line (BEBM) along with all the additives can be obtained from Lonza/Clonetics Corporation as a kit: BEGM, Kit Catalog No. CC-3170. ATCC does not use the GA-1000 (gentamycin-amphotericin B mix) provided with the BEGM kit. Note: Do not filter complete medium.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: BEAS-2B (ATCC® CRL-9609™)

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/bronchus
Disease: normal
Cell Type: epithelial (virus transformed)
Morphology: epithelial
Growth Properties: adherent
DNA Profile:
Amelogenin: XY
CSF1PO: 9, 12
D13S317: 13
D16S539: 12
D5S818: 12,13
D7S820: 10, 13
THO1: 7, 9.3
TPOX: 6, 11
vWA: 17, 18

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.
Note: The culture flasks used should be pre-coated with a mixture of 0.01 mg/mL fibronectin, 0.03 mg/mL bovine collagen type I and 0.01 mg/mL bovine serum albumin dissolved in BEBM medium (see references : U.S. Pat. 4,885,238 and Lechner, J.F. and LaVeck, M.A. A serum-free method for culturing normal human bronchial epithelial cells at clonal density. J. Tissue Culture Methods 9: 43-48, 1985).

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 centrifuge the cell suspension at approximately 125 x *g* for 5 to 10 minutes. Discard the supernatant.
4. Resuspend the cell pellet in the complete culture medium at the dilution ratio recommended in the specific batch information and dispense into a pre-coated T-25 cm^2 culture flask.
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.

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

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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 **pre-coated** 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

These cells should be **subcultured before reaching confluence** since confluent cultures rapidly undergo squamous terminal differentiation. Volumes used in this protocol are for 75 cm^2 flask; 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 0.25% Trypsin - 0.53mM EDTA solution containing 0.5% polyvinylpyrrolidone (PVP) to flask and observe cells under an inverted microscope until cell layer is dispersed (usually with 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. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

3. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
4. Transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes
5. Discard supernatant and resuspend cells in fresh growth medium. Inoculate new flasks at 1500 to 3000 cells per cm^2. The culture flasks used should be pre-coated with a mixture of 0.01mg/ml fibronectin, 0.03 mg/ml bovine collagen type I and 0.01 mg/mL bovine serum albumin dissolved in BEBM.
6. Place culture flasks in incubators at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in ***Culture of Animal Cells, a manual of Basic Technique*** by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.
Interval: Subcultured before reaching confluence.
Medium Renewal: Every 2 to 3 days

Flask Coating

1. Prepare a mixture of 0.01 mg/mL fibronectin, 0.03 mg/mL bovine collagen type I and 0.01 mg/mL bovine serum albumin (BSA) dissolved in culture medium. Store pre-prepared Coating Solution at 4°C in cold room for up to 3 months.
2. For a growth area of 75 cm^2, add 4.5 mL of the fibronectin/collagen/BSA solution and rock gently to coat the entire surface.
3. Incubate the freshly coated vessel(s) in a 37°C incubator overnight (it is preferable to use tissue culture vessels with tightened, plug-seal caps to prevent evaporation during the coating process).
4. Store coated flasks with solution at room temperature, light protected, up to 1 month. Suction off solution before plating cells.

Cryopreservation Medium

Cryoprotectant Medium
Complete growth medium plus 1% PVP and 7.5% DMSO.
Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Comments

The cells stain positively for keratins and SV40 T antigen.

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.

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Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. 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 strain. 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.
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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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