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BCRJ Code: 0395

Cell Line: BEAS-2B

Species: Homo sapiens

Vulgar Name: Human

Tissue: Lung, bronchus

Cell Type: Epithelial virus transformed

Morphology: Epithelial

Disease: Normal

Growth Properties: Adherent

Derivation: BEAS-2B cells were derived from normal bronchial epithelium obtained from autopsy of non-cancerous individuals. Cells were infected with a replication-defective SV40/adenovirus 12 hybrid and cloned. Squamous differentiation can be observed in response to serum. This ability can be used for screening chemical and biological agents inducing or affecting differentiation and/or carcinogenesis. The cell line has been applied for studies of pneumococcal infection mechanisms. BEAS-2B was described to express keratins and SV40 T antigen. Subculturing the cells before confluency is necessary as confluent cultures rapidly undergo squamous terminal differentiation. This material is cited in a U.S and / or other Patent (U.S. Pat. 4,885,238) and may not be used to infringe the patent claims.

Applications: This cell line is a suitable transfection host. The cells retain the ability to undergo squamous differentiation in response to serum, and can be used to screen chemical and biological agents for ability to induce or affect differentiation and/or carcinogenesis.

Tumor Formation:: The cells were not tumorigenic in immunosuppressed mice, but did form colonies in semisolid medium. Yes, the cells did form colonies in semisolid medium. No, the cells were not tumorigenic in immunosuppressed mice.

Biosafety: 2



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Culture 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

Subculturing:

These cells should be subcultured before reaching confluence since confluent cultures rapidly undergo squamous terminal differentiation. 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. Remove and discard culture medium. 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. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes Discard supernatant and resuspend cells in fresh growth medium. Inoculate new flasks at 1500 to 3000 cells per cm². 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. 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 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. For a growth area of 75 cm², add 4.5 mL of the fibronectin/collagen/BSA solution and rock gently to coat the entire surface. 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). Store coated flasks with solution at room temperature, light protected, up to 1 month. Suction off solution before plating cells.

Culture Conditions:

Atmosphere: air, 95%; carbon dioxide (CO₂), 5% Temperature: 37°C

Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)

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Thawing Frozen Cells:

SAFETY PRECAUTION: It is strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris.

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize 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 its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions.
3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately 125 × g for 5 to 7 minutes.
4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio).
5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line).

NOTE: It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at its normal pH (7.0 to 7.6).

References:

Reddel RR, et al. Immortalized human bronchial epithelial mesothelial cell lines. US Patent 4,885,238 dated Dec 5 1989 Lechner JF, LaVeck MA. A serum-free method for culturing normal human bronchial epithelial cells at clonal density. J. Tissue Culture Methods 9: 43-48, 1985. Sakamoto O, et al. Role of macrophage-stimulating protein and its receptor, RON tyrosine kinase, in ciliary motility. J. Clin. Invest. 99: 701-709, 1997. PubMed: 9045873 Hay RJ, Caputo JL, Macy, ML, Eds. (1992) ATCC Quality Control Methods for Cell Lines. 2nd edition, Published by ATCC. Caputo JL. Biosafety procedures in cell culture. J. Tissue Culture Methods 11:223-227, 1988 Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) Laboratory Safety: Principles and Practice. Second edition, ASM press, Washington, DC.

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