| | |
|---|---|
| **BCRJ Code:** | 0359 |
| **Cell Line:** | NCI-H441 [H441] |
| **Species:** | Homo sapiens |
| **Vulgar Name:** | Human |
| **Tissue:** | Lung |
| **Morphology:** | Epithelial |
| **Disease:** | Papillary adenocarcinoma |
| **Growth Properties:** | Adherent |
| **Sex:** | Male |
| **Applications:** | The line has been used as a transfection host for expression of pulmonary surfactant protein (SP-B). |
| **Biosafety:** | 1 |
| **Addtional Info:** | The cell line expresses mRNA and protein of the major surfactant apoprotein (SP-A). Electron microscopy shows multilamellar bodies and cytoplasmic structures resembling clara cell granules. The cells can be cloned in soft agar with or without serum. |
| **Culture Medium:** | RPMI-1640 medium modified to contain 2 mM L-glutamine, 4500 mg/L glucose with fetal bovine serum to a final concentration of 10%. |

| | |
|---|---|
| **Subculturing:** | Volumes used in this protocol are for 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum that contains trypsin inhibitor. Add 1.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until the 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. 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. Add appropriate aliquots of cell suspension to new culture vessels. An inoculum of 5 x 103 to 7 x 103 viable cells/cm2 is recommended. Place culture vessels in incubators at 37°C. Maintain cultures at a cell concentration between 3 x 10e4 and 5 x 10e4 cells/cm2. Population Doubling Time: 58 hrs in medium with serum NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010. |
| **Subculturing Medium Renewal:** | 2 to 3 times per week |
| **Subculturing Subcultivation Ratio:** | 1:3 to 1:8 |
| **Culture Conditions:** | Atmosphere: air, 95%; carbon dioxide (CO2), 5% Temperature: 37°C |
| **Cryopreservation:** | 95% FBS + 5% DMSO (Dimethyl sulfoxide) |

## Thawing Frozen Cells:

SAFETY PRECAUTION: Is highly recommend 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. 1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the Oring 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. For cells that are sensitive to DMSO is recommended that the cryoprotective agent be removed immediately. 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.Discard the supernatant and Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). 5. Incubate the culture in a appropriate atmosphere and temperature (see "Culture Conditions" for this cell line). NOTE: 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).

## References:

Bepler G, et al. Expression of p64c-myc and neuroendocrine properties define three subclasses of small cell lung cancer. Oncogene 4: 45-50, 1989. PubMed: 2536917 Brower M, et al. Growth of cell lines and clinical specimens of human non-small cell lung cancer in a serum-free defined medium. Cancer Res. 46: 798-806, 1986. PubMed: 3940644 Broers JL, et al. Spontaneous changes in intermediate filament protein expression patterns in lung cancer cell lines. J. Cell Sci. 91: 91-108, 1988. PubMed: 2473086 O'Reilly MA, et al. Differential effects of glucocorticoid on expression of surfactant proteins in a human lung adenocarcinoma cell line. Biochim. Biophys. Acta 970: 194-204, 1988. PubMed: 3382698 O'Reilly MA, et al. In vitro translation, post-translational processing and secretion of pulmonary surfactant protein B precursors. Biochim. Biophys. Acta 1011: 140-148, 1989. PubMed: 2713400 Gazdar AF, et al. Peripheral airway cell differentiation in human lung cancer cell lines. Cancer Res. 50: 5481-5487, 1990. PubMed: 2386953 Baatz JE, et al. Utilization of modified surfactant-associated protein B for delivery of DNA to airway cells in culture. Proc. Natl. Acad. Sci. USA 91: 2547-2551, 1994. PubMed: 8146151 Lung Cancer 4: 155-161, 1988. Tamura T, Stadtman TC. A new selenoprotein from human lung adenocarcinoma cells: purification, properties, and thioredoxin reductase activity. Proc. Natl. Acad. Sci. USA 93: 1006-1011, 1996. PubMed: 8577704 Yamaguchi Y, et al. Biochemical characterization and intracellular localization of the Menkes disease protein. Proc. Natl. Acad. Sci. USA 93: 14030-14035, 1996. PubMed: 8943055

| | |
|---|---|
| **Depositors:** | Rui Manoel Reis - Hospital de Câncer de Barretos |
| **ATCC:** | HTB-174 |