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: 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:

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

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