

## NCI-H446 Cells | 305049

## General information

**Description** This cell line was established in 1982 by D. Carney, A.F. Gazdar and associates from the pleural fluid of a patient with small cell cancer of the lung. The original tumor morphology was not characteristic of small cell lung cancer. The cell line is a variant small cell lung cancer in biochemistry and morphology, and expresses neuron specific enolase as well as the brain isoenzyme of creatine kinase. None of L-DOPA decarboxylase, bombesin, vasopressin, oxytocin or gastrin releasing peptide has been detected in the cell line. This cell line exhibits a 20-fold higher degree of c-myc DNA amplification and a 15-fold higher degree of c-myc RNA. The cell line was originally propagated in serum free RPMI 1640 medium supplemented with 10 nM of hydrocortisone, 5 microgram/mL of insulin, 10 microgram/mL of transferrin, 10 nM of 17-beta-estradiol, and 30 nM of sodium selenite. Transplantable tumors with non-typical small cell lung cancer histology can be formed by the cells.

**Organism** Human

**Tissue** Lung

**Disease** Lung small cell carcinoma

**Metastatic site** Pleural Effusion

**Synonyms** NCI-H446, H-446, NCI-446, NCIH446

## Characteristics

**Age** 61 years

**Gender** Male

**Ethnicity** European

**Morphology** Epithelial-Like

**Growth properties** Adherent

## Regulatory Data

**Citation** NCI-H446 (Cytion catalog number 305049)

**Biosafety level** 1

**NCBI\_TaxID** 9606

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CellosaurusAccession CVCL\_1562

**Biomolecular Data****Tumorigenic** Yes, in nude mice (The cells form transplantable tumors with non-typical SCLC histology).**Handling****Culture Medium** RPMI 1640, w: 2.0 mM stable Glutamine, w: 2.0 g/L NaHCO<sub>3</sub> (Cytion article number 820700a)**Supplements** Supplement the medium with 10% FBS, add 2.5 g/L glucose, 10 mM HEPES and 1.0 mM Sodium pyruvate**Dissociation Reagent** Accutase**Subculturing** Gather the suspension cells in a 15 ml tube and gently wash the adherent cells with PBS lacking calcium and magnesium (use 3-5 ml for T25 flasks and 5-10 ml for T75 flasks). Apply Accutase (1-2 ml for T25 flasks, 2.5 ml for T75 flasks) ensuring full coverage of the cell layer. Allow the cells to incubate at room temperature for 10 minutes. Following incubation, combine and centrifuge both the suspension and adherent cells. After centrifugation, carefully resuspend the cell pellet and transfer the cell suspension into new flasks containing fresh medium.**Fluid renewal** 2 to 3 times per week**Freeze medium** As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

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### Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below  $-150^{\circ}\text{C}$  to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a  $37^{\circ}\text{C}$  water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at  $300 \times g$  for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

### Incubation Atmosphere

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified atmosphere.

### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

### Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about  $-150$  to  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  is acceptable only as a short interim step before transfer to liquid nitrogen.

## Quality Control & Molecular Analysis

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**Sterility**

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.