

NCI-H460 Cells | 305020

**Description** NCI-H460, also known as H460, was derived from a male patient with large cell lung carcinoma. NCI-H460 cells are adherent cells growing twice as fast than the A549 cells with a doubling time of 33 hours in RPMI 1640 supplemented with 10% FBS. They can form tumors in both in vitro and in vivo models, including nude mice. NCI-H460 cells have been shown to express p53 mRNA at high levels comparable to normal lung tissue, while exhibiting no gross structural DNA abnormalities. They stain positively for keratin and vimentin but are negative for neurofilament triplet protein. Isoenzyme analysis has shown that HPRT is localized on the surface of these non-small-cell lung cancer cell lines. AK-1, ES-D, and Me-2 isoenzymes are expressed at level 1, while G6PD and PGM1 and PGM3 isoenzymes are expressed at level B and 1-2, respectively. The cells have a hypotriploid karyotype with a modal chromosome number of 57, ranging from 53 to 65. Seven marker chromosomes are common to all cells, including der(9)t(1;9)(q21;p24), der(9)t(7;9)(p11;p22), t(10q14q), der(16)t(7;16)(q11.23;q22). Their high expression levels of p53 mRNA make them a suitable model for studying the molecular mechanisms of non-small-cell lung cancer.

**Organism** Human

**Tissue** Lung

**Disease** Lung large cell carcinoma

**Metastatic site** Pleural effusion

**Synonyms** NCI-H460, NCI.H460, H-460, NCIH460, NCI-HUT-460, NCI-460

**Gender** Male

**Ethnicity** European

**Morphology** Epithelial

**Growth properties** Adherent

**Citation** H-460 (Cytion catalog number 305020)

**Biosafety level** 1

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**NCBI\_TaxID** 9606**CellosaurusAccession** CVCL\_0459**Tumorigenic** Yes**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**Dissociation Reagent** Accutase**Subculturing** Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain 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.

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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.