SOP: **Propagation of NCI-H460, Human Large Cell Lung Carcinoma Cells**
Date modified: **03/27/2013**
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Ordering Information

Human Large Cell Lung Carcinoma Cells NCI-H460 can be ordered from ATCC as a frozen ampoule with 1.5 x 10^6 cells per 1mL volume. This is an adherent cell line.

Name: NCI-H460—Human Large Cell Lung Carcinoma Cells
ATCC #: HTB-177

Materials List

1. RPMI-1640 Medium (1X) with L-glutamine (Corning/Cellgro, Cat# 10-040-CV)
2. Characterized Fetal Bovine Serum (HyClone, Cat# SH30071)
3. Penicillin-Streptomycin Solution (200X) (Corning/Cellgro, Cat# 30-001-CI)
4. D-(+)-Glucose Solution (45%) (Sigma-Aldrich, Cat# G8769)
5. HEPES Buffer, 1M Solution (Corning/Cellgro, Cat# 25-060-CI)
6. Sodium Pyruvate, 100mM Solution (Corning/Cellgro, Cat# 25-000-CI)
7. T25, T75, T225 tissue culture flasks
8. Corning conical centrifuge tubes (15mL and 50mL)
9. Graduated pipets (1, 5, 10, 25, 50mL)
10. Phosphate Buffered Saline (1X PBS) (Corning/Cellgro, Cat# 21-040-CM)
11. Accutase Enzyme Cell Detachment Medium (EBiosciences, Cat# 00-4555)
12. Freezing Medium (Growth medium containing 5% DMSO)
13. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
14. Cryovials (Nunc, Cat# 368632)
15. Cryo 1°C Freezing Container (Nalgene Cat# 5100-0001)
16. Eppendorf Centrifuge 5810R
17. Revco UltimaII -80°C Freezer
18. Thermolyne Locator 4 Liquid Nitrogen Freezer
19. Hemocytometer
20. Micropipet w/ P20 tips
21. Microscope

Growth Medium for NCI-H460

RPMI-1640 with L-glutamine Medium
10% Characterized FBS
Pen-Strep (1X)
4.5g/L D-Glucose
10mM HEPES Buffer
1mM Sodium Pyruvate

Procedure

A. Receipt of Frozen Cells and Starting Cell Culture

1) Immediately place frozen cells in liquid nitrogen storage until ready to culture.
2) When ready to start cell culture, quickly thaw ampoule in a 37°C water bath.
3) As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol, then dispense contents of ampoule into a 15mL Corning centrifuge tube containing 9mL complete culture medium.
4) Pellet cells at 125 x g for 7 minutes (4°C).
5) Resuspend cell pellet in 20mL complete culture medium and dispense into a T75 flask.
6) To incubate the culture, place the flask in a 37°C, 5% CO_2 humidified incubator.

B. Sub-culture

1) Propagate cells until density reaches 70-80% confluence.
2) Aspirate medium.
3) Wash cells with warm 1X PBS.
4) Add 15mL of Accutase and return to incubator for 10-15 minutes, or until cells detach.
5) Immediately remove cells, rinse flask with warm 1X PBS to collect residual cells, and pellet at 500 x g for 5 minutes (4°C).
6) Gently re-suspend cell pellet in warm medium.
7) Perform 1:3 to 1:8 cell split as needed.
8) Record each subculture event as a passage.

C. Maintenance and Generation of Seed Stocks

1) Change media the day after seeding and every 2-3 days thereafter. Use 50mL of growth medium per T225 flask.
2) Following first or second passage after receipt of cells and with sufficient number of cells to continue maintenance and expansion, the major portion of the flasks should be sub-cultured using Accutase as above under “Sub-culture” and a small portion should be set aside as a seed stock. The cell pellet for the seed stock should be resuspended in freezing medium.
3) Cells in freezing medium are dispensed into cryovials (2 million cells per 1mL aliquot) and frozen at -80°C in a Nalgene Cryo 1°C freezing container overnight.
4) Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

D. Harvest

1) Passage cells until the desired number of cells is reached.
2) Remove cells from flasks according to protocol described above under “Sub-culture”.
3) Examine viability using Trypan blue staining (SOP TP-7).