SOP: **Propagation of NCI-H226 Human Lung Mesothelioma Cells**
Date modified: **7/13/2012**
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Ordering Information

NCI-H226 Human Lung Mesothelioma Cells can be ordered from ATCC as a frozen ampoule with 0.9 x 10^6 cells per 1mL volume. This is an adherent metastatic squamous cell carcinoma epithelial cell line from a human male.

Name: NCI-H226—Human Lung Mesothelioma Cells
ATCC #: CRL-5826

Materials List

1. RPMI-1640 Medium (ATCC, Cat# 30-2001)
2. Characterized Fetal Bovine Serum (HyClone, Cat# SH30071)
3. Penicillin-Streptomycin Solution (200X) (Cellgro, Cat# 30-001-CI)
4. T25, T75, T225 tissue culture flasks
5. Corning conical centrifuge tubes (15mL and 50mL)
6. Graduated pipets (1, 5, 10, 25, 50mL)
7. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
8. Accutase Enzyme Cell Detachment Medium (EBiosciences, Cat# 00-4555)
9. Freezing Medium (Growth medium containing 5% DMSO)
10. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
11. Cryovials (Nunc, Cat# 368632)
12. Cryo 1^{o}C Freezing Container (Nalgene Cat# 5100-0001)
13. Eppendorf Centrifuge 5810R
14. Revco UltimaII -80^{o}C Freezer
15. Thermolyne Locator 4 Liquid Nitrogen Freezer
16. Hemocytometer
17. Micropipet w/ P20 tips
18. Microscope

Growth Medium for NCI-H226

RPMI-1640 Medium
10% Characterized FBS
Pen-Strep (1X)

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^{o}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^{o}C).
5) Resuspend cell pellet in 10mL complete culture medium and dispense into a T25 flask.
6) To incubate the culture, place the flask in a 37^{o}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 room temperature 1X PBS.
4) Add 5mL of Accutase and return to incubator for 5-10 minutes, or until cells detach.
5) Immediately remove cells, rinse flask with room temperature 1X PBS to collect residual cells, and pellet at 300 x g for 5 minutes (4^{o}C).
6) Gently re-suspend cell pellet in prewarmed medium.
7) Perform 1:4 to 1:8 cell split as needed.
8) Record each subculture event as a passage.
9) Note: use increasing amounts of Accutase with increasing sizes of tissue culture vessel to sub-culture.

C. Maintenance and Generation of Seed Stocks

1) Change media the day after seeding and every 2-3 days thereafter. Use 10mL of growth medium per T25 flask, 20mL of growth medium per T75 flask, and 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 1 mL aliquot) and frozen at -80^{o}C in a Nalgene Cryo 1^{o}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", using 15mL of Accutase per T225 flask.
3) Examine viability using Trypan blue staining (SOP TP-7).