

**SOP:** Propagation of A549, Human Lung Carcinoma Epithelial Cells  
**Date modified:** 10/6/2010  
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### **Ordering Information**

A549 can be ordered from ATCC as a frozen ampoule. This is an adherent cell line.

Name: A549—Human Lung Carcinoma Epithelial Cells  
ATCC #: CCL-185

### **Materials List**

1. F-12K Medium (ATCC, Cat# 30-2004)
2. Characterized Fetal Bovine Serum (HyClone, Cat# SH30071)
3. T25, T75, T225 tissue culture flasks
4. Corning conical centrifuge tubes (15mL and 50mL)
5. Graduated pipets (1, 5, 10, 25, 50mL)
6. Penicillin-Streptomycin Solution (200X) (Cellgro, Cat# 30-001-CI)
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°C Freezing Container (Nalgene, Cat# 5100-0001)
13. Eppendorf Centrifuge 5810R
14. Revco UltimaII -80°C Freezer
15. Thermolyne Locator 4 Liquid Nitrogen Freezer
16. Hemocytometer
17. Micropipet w/ P20 tips
18. Microscope

### **Growth Medium for A549**

F-12K Medium  
10% 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°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 T25 flask with 10mL of warm growth media. Cultures can be established between  $2 \times 10^3$  and  $1 \times 10^4$  viable cells/cm<sup>2</sup>. Do not exceed  $7 \times 10^4$  cells/cm<sup>2</sup>.
- 4) Allow cells to recover overnight in 37°C, 5% CO<sub>2</sub> humidified incubator.
- 5) The next morning, the diluted DMSO-containing shipping/cryopreservation medium is aspirated from the cell layer and replaced with fresh medium.

#### **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) Maintain cultures at a cell concentration between  $6 \times 10^3$  and  $6 \times 10^4$  cells/cm<sup>2</sup>.
- 2) Change media the day after seeding and every 2-3 days thereafter. Use 50mL of growth medium per T225 flask.
- 3) 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.
- 4) Cells in freezing medium are dispensed into cryovials (2 million cells per 1 mL aliquot) and frozen at -80°C in a Nalgene Cryo 1°C freezing container overnight.
- 5) 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).