

SOP: Propagation of A549 (ATCC CCL-185)

Information

Name: A549
ATCC #: CCL-185
Tissue: lung
Product Format: frozen
Morphology: epithelial
Culture Properties: adherent
Biosafety Level: 1
Disease: carcinoma: 58-year-old Caucasian male
Population Doubling Time: about 22 hours

Materials List

1. F-12K (Fisher; MT10-025-CV)
2. Heat Inactivated Fetal Bovine Serum (Life Technologies; Cat# 10082147)
3. Penicillin-Streptomycin 10,000U/mL (Life Technologies; Cat#15140)
4. Phosphate Buffered Saline (1X PBS) w/o Ca^{2+} , Mg^{2+} (CORNING Cellgro; Cat# 21-040-CM)
5. TrypLE Express (Life Technologies; Cat#12604)
6. T75, T525 culture flasks
7. Graduated pipets (1, 5, 10, 25, 50 mL)
8. Freezing medium (growth medium containing 95%; DMSO, 5%)
9. DMSO (Fisher; Cat#BP-231-100)
10. Cryovials (Sarstedt; Cat #72-694-006)
11. TC20 cell counter (Bio-Rad)
12. Counting Slides (Bio-Rad; Cat# 145-0011)
13. Microscope

Growth Medium for A549

F-12K
10% FBS
Pen-Strep (1X)

Procedure

A. Receipt of Frozen Cells and Starting Cell Culture

- 1) Immediately place frozen cells in liquid nitrogen freezer 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 T75 flask with 20 ml of warm growth media.
- 4) Allow cells to recover overnight in 37°C, 5% CO₂ 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

Volumes used in this protocol are for 75 cm² and/or 525 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of the other size.

- 1) Propagate cells until density reaches 70-80% confluence.
- 2) Aspirate medium.
- 3) Wash cells with room temperature (or warm) 1X PBS
- 4) Add 3 mL (T-75) or 30 mL (T525) of TrypLE and return to incubator for 3 minutes, or until cells detach.
- 5) Add 7 ml (T-25) or 30 mL (T525) of complete growth medium and aspirate the cells by gentle pipetting.
- 7) Perform 1:3 to 1:8 cell split as needed.

Note: Cultures can be established between 2×10^3 and 1×10^4 viable cells/cm²8)

- 8) Incubate cultures at 37°C, 5% CO₂ humidified incubator.
- 9) Change Medium 2 to 3 times per week.
- 10) Record each subculture event as a passage.

C. Maintenance and Generation of Seed Stocks

- 1) Change media the day after seeding and every 3-4 days thereafter. Use 100 mL of growth medium per T525 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 TrypLE 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 (1-2 million cells per 1mL aliquot) and frozen in a -80°C cryo-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 as described above under “Sub-culture”.
- 3) Examine viability using Trypan blue staining.