Protocol: Cell Culturing – A549 Adenocarcinomic Human Alveolar Basal Epithelial Cells

Protocol Notes:

- This protocol was modified from: "Cell Growth Protocol for A549 Cell Line"[1]

Biohazard Safety:

- Be sure to work in a biohazard safety cabinet at all times. Wipe the cabinet before and after use with 70% ethanol or Bacdown disinfectant.

- Use universal biosafety precautions, wear a lab coat and disposable gloves, and other appropriate PPE.
- When changing growth medium, add bleach to the discarded media for 10% bleach solution to disinfect the cells and media for at least 10 minutes. Discard and rinse down the drain.
- While spinning in centrifuge, cover the samples with the "Biohazard" caps to prevent the spread of infectious content in the case of a spill.
- Dispose trash in the designated "Biohazard" trashcans. Be sure to double bag the pipets before disposal.

Materials:

1. 15-mL polystyrene conical tubes
2. 50-mL polystyrene conical tubes
3. Cryogenic Vials, Corning Incorporated, 2-mL, Lot# 34308068, Cat# 430488,
4. T-75 cm2 Flask
    - Thermo Scientific, BioLite 75 cm2 Flask Vented, Lot#A5RA5NG113, Cat#130190
5. T-175 cm2 Flask

[1] Authors: Created by SPoynter on 2012-07-31; Modified by SP on 2012-08-12; Modified by PInjean 2013-01-01; Modified by RKern 2013-02-01

- Thermo Scientific, BioLite 175 cm2 Flask Vented, Lot#C4WA4PE112, Cat#130191

6. A549 Cell Line, Location: 1st floor freezer
   - Frozen at P20 by N/A on N/A
   - Thawed on 2012-07-30 by SP
7. Growth Medium
   - DMEM: HyClone, Cat# SH30285.01, Lot# AXF39350, Expiration: Dec. 2012
   - Fetal Bovine Serum (FBS): Prepared by N/A on N/A
   - Pen/Strep: Prepared by N/A on N/A, Location: refrigerator #5
   - Amp B: Prepared by N/A on N/A, Location: refrigerator #5
   - HEPES: Cellgro, Cat# 25-060-Cl, Lot# 25060106, Expiration: Sept. 2012
8. Trypsin/EDTA, prepared on N/A by N/A, location: refrigerator #6, -20 degrees C
9. Phosphate Buffered Saline (PBS) 1x, prepared on N/A by N/A
10. Freeze down solution, 90% (v/v) FBS, 10% (v/v) Dimethyl Sulfoxide (DMSO)
    - Fetal Bovine Serum (FBS): Prepared by N/A on N/A
    - Dimethyl Sulfoxide, Research Organics, Cat# 2166D, Lot# A85277, Received: Jan. 2010, Location: cell culturing room
11. Jouan CR 412 Centrifuge
12. Incubator

Methods:

Thawing Cells

1. Pipet sufficient volume of growth medium into 50-mL conical vial(s) and place medium into 37 C/5% CO_2 incubator for at least 30 minutes before use.
   - Calculation for medium volume:

2. Pipet 1-mL of warm growth medium into a 15-mL conical vial. Thaw cells in 37 C water bath until cell/ice pellet loosens from tube wall and quickly transfer cell/ice pellet to 1-mL of warm growth medium. Add 9-mL of warm growth medium to vial for total volume of approximately 11-mL. Gently invert tube several times.

3. Remove 20-uL of cells in growth medium and pipet into 0.5-mL microcentrifuge tube for cell count

4. Add 20-uL of Trypan Blue to the 20-uL aliquot of cells and mix well with pipet. Add 10-uL of mixture into cell counter.

    - Record cell counts and show calculations:

5. After counting cells, centrifuge original cell volume at 1200 RPM at 4 C for 5 minutes.

6. Remove medium from cells being careful not to disturb the cell pellet and add appropriate volume of fresh warm growth medium. Cells should be seeded between $2x10^3$ and $1x10^4$ cells/cm2. Flask size used will be determined on cell count, but a T-75 should get ~$5x10^5$ cells and a T-175 should get ~$1x10^6$ cells.

7. Change medium every 2-3 days and split confluent cultures 1:4 to 1:9 every 4 to 7 days.

Splitting Cells

1. Pipet sufficient volume of growth medium into 50-mL conical vial(s) and place medium into 37 C/5% CO_2 incubator for at least 30 minutes before use. A vial of trypsin/EDTA must also be thawed in preparation for splitting cells and should be placed in the incubator with the medium after thawing.

2. Remove growth medium from cell culture flasks with a sterile pipet and suction apparatus. Add trypsin/EDTA to each flask and roll solution back and forth along surface of flask for a few minutes to aid in loosening the cells. After agitating place flasks in incubator. Total trypsinization process should not take longer than 5-7 minutes.

3. Add warm growth medium (three times the volume of trypsin/EDTA used to lift the cells) to the flask and wash the medium over the complete flask surface several times to recover the cells.

4. Pipet cells into a conical vial and centrifuge at 1200 RPM at 4 C for 5 minutes.

5. Remove medium from cells being careful not to disturb the cell pellet and add appropriate volume of fresh warm growth medium. Cells should be seeded between $2x10^3$ and $1x10^4$ cells/cm2. Flask size used will be determined on cell count, but a T-75 should get ~$5x10^5$ cells and a T-175 should get ~$1x10^6$ cells. Record new cell passage number on new flask.

Freezing Cells

1. Spin resuspended cells at 1200 RPM for 5 minutes at 4 C.
2. Remove medium from cells being careful not to disturb the cell pellet and add appropriate volume of cold freeze down solution to obtain 2 or 3 million cells per cryogenic vial. Be sure to properly mix resuspended cells to ensure proper freeze down concentration.
3. Aliquot 1-mL of cells in freeze down solution in cryogenic vial(s). Work quickly as DMSO is toxic to cells.
4. Place cryogenic vial(s) in -80 C freezer tray overnight.
5. Move cryogenic vial(s) to -150 C freezer for storage the following day. Be sure to update the record folder.

References

1. "Cell Growth Protocol for A549 Cell Line" from Hudson Alpha/Caltech ENCODE group, prepared by Norma Neff and Tim Reddy http://a549.com/a549_cell_culture.html