

**SOP:** Propagation of Mouse NIH-3T3 Embryonic Fibroblasts  
**Date modified:** 01/18/2011  
**Modified by:** T. Canfield (UW)

### **Ordering Information**

Mouse NIH-3T3 Embryonic Fibroblasts can be ordered from ATCC as a frozen ampoule. This is an adherent mouse cell line.

Name: NIH-3T3—Mouse Embryonic Fibroblasts  
ATCC #: CRL-1658

### **Materials List**

1. DMEM (Dulbecco's Modification of Eagle's Medium) with 4.5g/L glucose, L-glutamine, and sodium pyruvate (Cellgro, Cat# 10-013-CV)
2. Bovine Calf Serum (Colorado Serum Company, Cat# CS1332)
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 Mouse NIH-3T3 Cells**

DMEM with 4.5g/L glucose, L-glutamine, and sodium pyruvate Medium  
10% Bovine Calf Serum  
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, and then transfer the vial contents to a 15mL conical centrifuge tube containing 9mL of complete growth medium.
- 4) Centrifuge at 125 x g (4°C) for 7 minutes.
- 5) Resuspend the cell pellet in complete growth medium at a cell concentration of  $5 \times 10^3$  cells/cm<sup>2</sup> in a T25 flask.
- 6) Incubate cells in 37°C, 5% CO<sub>2</sub> humidified incubator.

**B. Sub-culture**

- 1) Propagate cells until density reaches 70-80% confluence. **DO NOT ALLOW THE CELLS TO BECOME CONFLUENT.**
- 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) Inoculate larger flask(s) at  $3\text{-}5 \times 10^3$  cells/cm<sup>2</sup> as needed, subculturing about every 3 days.
- 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 1 mL 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 for experimentation.
- 2) Remove cells from flasks according to protocol described above under “Sub-culture” section.
- 3) Examine viability using Trypan blue staining (SOP).