SOP: **Propagation of Mouse MEL cells**
Date modified: **01/18/2011**
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Ordering Information

Mouse MEL cells were received from Dr. Sherman Weissman, Yale School of Medicine, New Haven, CT.

Notes:
This is a mouse suspension cell line.

Materials List

1. RPMI 1640 with 2mM L-glutamine Medium (Cellgro, Cat# 10-040-CM)
2. Characterized Fetal Bovine Serum (HyClone, Cat# SH-30071-03)
3. Penicillin-Streptomycin Solution, 200X (Cellgro, Cat# 30-001-CI)
4. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
5. T75, T225 tissue culture flasks
6. Corning conical centrifuge tubes (15mL and 50mL)
7. Graduated serological pipets (1, 5, 10, 25, 50mL)
8. Freezing Medium (90% Characterized Fetal Bovine Serum + 10% DMSO)
9. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
10. CryoVials (Nunc, Cat# 368632)
11. Cryo 1°C Freezing Container (Nalgene, Cat# 5100-0001)
12. Eppendorf Centrifuge 5810R
13. Revco UltimaII -80°C Freezer
14. Thermolyne Locator 4 Liquid Nitrogen Freezer
15. Hemocytometer
16. Micropipet w/ P20 tips
17. Microscope

Growth Medium for Mouse MEL Cells

RPMI 1640 with 2mM L-glutamine Medium
10% Characterized FBS
Pen-Strep (1X)

Procedure

A. Receipt of Frozen Cells and Starting Cell Cultures

1. Immediately place frozen cells in liquid nitrogen storage until ready to culture.
2. When ready to start cell culture, quickly thaw ampoule in 37°C water bath until ice crystals disappear.
3. Swab outside of the ampoule with 70% ethanol and then dispense contents of ampoule into a 15mL Corning conical centrifuge tube.
4. Add 10mL cold growth medium, drop wise, into the centrifuge tube containing cells.
5. Pellet cells gently at 200 x g 4°C 5 minutes and remove DMSO-containing supernatant.
6. Resuspend pellet at $2.5x10^5$cells/mL with pre-warmed growth medium and grow in a 37°C, 5% CO_2 humidified incubator. **Concentration of cells should never exceed $1x10^6$cells/mL**.

B. Sub-culture and Maintenance

1. Take cell counts with a hemocytometer every 24-48 hours to maintain the culture at a cell density between $1x10^5$cells/mL and $5x10^6$cells/mL.
2. Add fresh warm medium when appropriate to maintain cell density and expand the culture to the desired number of cells for generation of seed stock and experimentation.
3. Record each subculture event as a passage.

C. Generation of Seed Stocks

1. At an early stage of expansion and with sufficient number of cells to continue maintenance, a small portion of the cells should be set aside as a seed stock, if needed.
2. Amount of cells for the seed stock should be placed in a conical centrifuge tube and centrifuged at 500 x g (4°C) for 5 minutes.
3. Aspirate supernatant and resuspend the cell pellet in 1X PBS to wash. Centrifuge again under same conditions.
4. Resuspend the cell pellet in freezing medium (90% Characterized Fetal Bovine Serum + 10% DMSO) at a concentration yielding 5-10 million cells per 1mL aliquot.
5. Dispense 1mL cell suspension per cryovial. Place cryovials in a Nalgene Cryo 1°C freezing container and store overnight at -80°C.
6. 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 for experimentation is reached in a logarithmic growth phase.
2. Pellet cells and rinse with 1X PBS as in "Generation of Seed Stocks" section.
3. Examine viability using Trypan blue staining (SOP TP-7).