SOP: **Propagation of Mouse 3134 Mammary Adenocarcinoma Cells**
Date modified: **03/01/2011**
Modified by: **S. John (NIH), T. Canfield (UW)**

Ordering Information

Mouse 3134 is a murine mammary adenocarcinoma cell line generated in Dr. Gordon Hager's laboratory at the National Institutes of Health, Bethesda, MD.

Notes:
This adherent cell line contains a multi-copy, single integration of the mouse mammary tumor virus LTR (MMTV-LTR) driving the Ras gene on chromosome 4. The MMTV-LTR is hormone-responsive and is activated by a variety of steroid hormones. More information on this cell line can be obtained from Ostrowski, MC et al *Molecular Cell Biology* 1983 November; 3(11): 2045-2057.

Materials List

1. DMEM, 1X, with High Glucose (Invitrogen, Cat# 11960)
2. Fetal Bovine Serum (Sigma-Aldrich, Cat# F0926)
3. Charcoal Stripped Serum (Invitrogen, Cat# 12676)
4. L-Glutamine, 100X Solution (Invitrogen, Cat# 25030)
5. Non-essential Amino Acids, 100X Solution (Invitrogen, Cat# 11140-050)
6. Sodium Pyruvate, 100X Solution (Invitrogen, Cat# 111360)
7. Penicillin-Streptomycin Solution, 100X (Invitrogen, Cat# 15140)
8. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
9. Trypsin-EDTA (Invitrogen, Cat# 25200)
10. Dexamethasone (Sigma-Aldrich, Cat# D4902)
11. Alcohol, 200 proof (Decon Laboratories Inc., Cat# 2716)
12. T75, T225 tissue culture flasks
13. 150mm tissue culture dishes
14. Corning conical centrifuge tubes (15mL and 50mL)
15. Graduated serological pipets (1, 5, 10, 25, 50mL)
16. Freezing Medium (growth medium containing 10% DMSO)
17. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
18. CryoVials (Nunc, Cat# 368632)
19. Cryo 1^{o}C Freezing Container (Nalgene, Cat# 5100-0001)
20. Eppendorf Centrifuge 5810R
21. Revco UltimaII -80°C Freezer
22. Thermolyne Locator 4 Liquid Nitrogen Freezer
23. Hemocytometer
24. Micropipet w/ P20 tips
25. Microscope

Growth Medium for Mouse 3134 Cells

DMEM, 1X, with High Glucose
10% FBS
L-Glutamine (1X)
Non-essential Amino Acids (1X)
Sodium Pyruvate (1X)
Pen-Strep (1X)

Charcoal Stripped Medium for Hormone Treatment of Mouse 3134 Cells

DMEM, 1X, with High Glucose
10% Charcoal Stripped Serum
L-Glutamine (1X)
Non-essential Amino Acids (1X)
Sodium Pyruvate (1X)
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^{o}C water bath until ice crystals disappear.
3. Swab outside surface of the ampoule with 70% ethanol and then dispense contents of ampoule into a T75 tissue culture flask with 20mL of warm growth medium.
4. Allow cells to recover overnight in a 37°C, 5% CO_2 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. Mouse 3134 cells can be grown into confluency without affecting subsequent passaging.
2. Aspirate medium.
3. Wash cell layer with warm 1X PBS.
4. Add 5mL of Trypsin-EDTA and leave at room temperature for 5 minutes, or until cells detach.
5. Immediately remove detached cells to a centrifuge tube, 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 growth medium.
7. Perform a 1:10 cell split as needed. Alternatively, trypsinized cells can be passaged directly by adding to fresh medium (no centrifugation necessary).
8. Record each subculture event as a passage.

C. Maintenance and Generation of Seed Stocks

1. Change medium 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 1mL 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 at confluency.
2. Remove cells from flasks as described above under “Sub-culture.”
3. Examine viability using Trypan blue staining (SOP TP-7).

E. Hormone Treatment of Cells

1. Cells are split into 150mm tissue culture dishes at a concentration of 2 million cells per dish in charcoal stripped medium.
2. Cells are maintained in charcoal stripped medium for 2 days prior to hormone treatment.

3. Hormone treatments with corticosteroids such as dexamethasone are for 1 hour with 100nM corticosteroid. Dexamethasone is reconstituted to a stock concentration of 20µg/mL following manufacturer's specifications.