SOP: **Propagation of Mouse MEL-GATA-1-ER cells**
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Ordering Information

Mouse MEL-GATA-1-ER cells (mG/ER) were received from Dr. Arthur Skoultchi, Albert Einstein College of Medicine of Yeshiva University, Bronx, New York.

Notes:
This is a mouse suspension cell line derived from MEL cells by stable transfection with a GATA-1-ER fusion protein construct as described by Choe et al., 2003 (Cancer Res 63, 6363–6369, 2003). These cells can be terminally differentiated into mature erythroid cells with β-estradiol treatment.

Materials List

1. DMEM, 1X, Dulbecco's Modification of Eagle's Medium, with 4.5g/L glucose, L-glutamine, sodium pyruvate (Cellgro, Cat# 10-013-CV)
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. Puromycin dihydrochloride (Sigma-Aldrich, Cat# P8833)
6. β-Estradiol (Sigma-Aldrich, Cat# E2257)
7. T75, T225 tissue culture flasks
8. Corning conical centrifuge tubes (15mL and 50mL)
9. Graduated serological pipets (1, 5, 10, 25, 50mL)
10. Freezing Medium (growth medium containing 10% DMSO)
11. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
12. CryoVials (Nunc, Cat# 368632)
13. Cryo 1°C Freezing Container (Nalgene, Cat# 5100-0001)
14. Eppendorf Centrifuge 5810R
15. Revco UltimaII -80°C Freezer
16. Thermolyne Locator 4 Liquid Nitrogen Freezer
17. Hemocytometer
18. Micropipet w/ P20 tips
19. Microscope

Growth Medium for Mouse G/ER Cells

DMEM, 1X, with 4.5g/L glucose, L-glutamine, sodium pyruvate
10% Characterized FBS
Pen-Strep (1X)
Puromycin dihydrochloride (5µg/mL)

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 $2x10^5$cells/mL with pre-warmed growth medium and grow in a 37°C, 10% 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 hours to maintain the culture at a cell density between $2x10^5$cells/mL and $1x10^6$cells/mL. The cells have a doubling time of 12 hours and the concentration of cells should not exceed $1x10^6$cells/mL.
2. Add fresh warm medium when appropriate to maintain cell density and expand the culture to the desired number of cells.
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 (growth medium containing 10% DMSO) at a concentration yielding 2 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).

E. Differentiation

1. The cells can be differentiated to red blood cells by adding β-estradiol to the growth medium to 10^{-7}M final concentration (freshly thawed 10^{-4}M stock from -20°C storage, made according to Sigma-Aldrich specifications for solubilization and storage). The cells are committed to differentiation within 48 hours; within 120 hours, 95% of cells are differentiated.