SOP: **Propagation of ELF-1 Human Embryonic Stem Cell Line**
Date modified: **04/02/2013**
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Cell Line Information

Human embryonic stem cell line ELF-1 was derived from a human embryo in the Ellison Stem Cell Core at the University of Washington, Seattle, WA. [NIH Stem Cell Registry (0156); will be banked at WiCell for distribution].

Materials List

1. DMEM/F12 Media (1X), liquid 1:1 (Invitrogen, Cat# 11320-082)
2. Knockout Serum Replacer (Invitrogen, Cat# 10828-028)
3. MEM Sodium Pyruvate Solution, 100X (Invitrogen, Cat# 11360-070)
4. Non-essential Amino Acids, 100X (Invitrogen, Cat# 11140-050)
5. Penicillin/Streptomycin, liquid (Invitrogen, Cat# 15070-063)
6. 2-Mercaptoethanol (Sigma-Aldrich, Cat# M7522)
7. Dulbecco's Modified Eagle Medium (DMEM) (1X), liquid (high glucose), GlutaMAX™ (Invitrogen, Cat# 10566-024)
8. Fetal Bovine Serum (Atlanta Biologicals, Cat# S10250)
9. Gelatin from porcine skin Type A, powder, cell culture tested (Sigma-Aldrich, Cat# G1890)
10. BD Matrigel™ Basement Membrane Matrix, Growth Factor Reduced (GFR) (BD, Cat# 354230)
11. Human LIF (Millipore, Cat# LIF1010)
12. MEK Inhibitor (Selleck Chemicals, Cat# S1036)
13. GSK3 Inhibitor (Selleck Chemicals, Cat# S1263)
14. 35mm and 10cm Tissue Culture Dishes
15. Conical Polypropylene Centrifuge Tubes (15mL and 50mL)
16. Graduated Serological Pipets (1, 5, 10, 25, 50mL)
17. Dulbecco's Phosphate-Buffered Saline (D-PBS) 1X, liquid (Invitrogen, Cat# 14190-250)
18. 0.05% Trypsin-EDTA (1X), Phenol Red (Invitrogen, Cat# 25300-120)
19. Accutase Enzyme Cell Detachment Medium (EBiosciences, Cat# 00-4555)
20. cOmplete™, EDTA-free, Protease Inhibitor Cocktail Tablets (Roche Diagnostics Corp., Cat# 05056489001); use 1 tablet per 50mL solution.
21. Freezing Medium (60% Growth Medium, 30% Knockout Serum Replacer, 10% DMSO)
22. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
23. Freezing Straws (Veterinary Concepts, Cat# 04170)
24. Gamma-irradiated MEF Cells (generated in the Ellison Stem Cell Core, and frozen)
25. Beckman Coulter Allegra X-22 Centrifuge
26. Bio-Cool Controlled Rate Freezer
27. New Brunswick Cell Counter
28. Scissors
29. Microscope

Growth Medium for ELF-1 Cells

500mL DMEM/F12 (1:1) Medium
100mL Knockout Serum Replacer
6mL Pen/Strep
6mL MEM Sodium Pyruvate Solution
6mL NEAA (Non-essential Amino Acids)
0.6mL 0.1M 2-Mercaptoethanol
0.8µM MEK Inhibitor
1.5µM GSK3 Inhibitor
10µg/mL Human LIF

Freezing Medium for ELF-1 Cells

3mL Growth Medium
1.5mL Knockout Serum Replacer
0.5mL DMSO

Plating Medium for Irradiated MEF Cells

500mL DMEM GlutaMAX™ (High Glucose) Medium
60mL Fetal Bovine Serum
6mL Pen/Strep

Procedure

A. Thawing Frozen Cells and Starting Cell Culture

1) When ready to start cell culture, quickly thaw 1 straw in a small container of room temperature water.
2) After about 10 seconds, spray straw and a pair of scissors with 70% ethanol, then dispense contents of straw by cutting the top and bottom off and allowing contents to empty into a 15mL conical centrifuge tube containing 8mL complete culture medium.
3) Pellet cells at 300 x g for 3 minutes.
4) Re-suspend cell pellet in 4mL complete culture medium and dispense into two 35mm tissue culture dishes with 1.5 x 10^5 irradiated MEFs (9.0 x 10^5 irradiated MEFs on 10cm tissue culture dish), plated overnight on 0.1% gelatin-coated tissue culture dishes.
5) To culture, place the dish in a 37°C, 5% O_2, 5% CO_2, 90% N_2, humidified incubator.

B. Sub-culture

1) Propagate cells for 3-4 days, changing medium every 1-2 days.
2) Aspirate medium.
3) Wash cells with 1X PBS.
4) Add 1.5mL of Trypsin to 35mm tissue culture dish (3mL for 10cm tissue culture dish) and let sit at room temperature for 5-10 minutes, or until cells detach.

5) Immediately remove cells, rinse tissue culture plate with equal amount of growth medium to collect residual cells, and pellet at 300 x g for 3 minutes.
6) Gently re-suspend cell pellet in growth medium.
7) Perform 1:5 to 1:10 split every 3-4 days.

C. Harvest

1) Passage cells until the desired number of cells is reached. On the last passage before collection, plate cells on Matrigel (diluted 1:30 in PBS) coated tissue culture dishes.
2) At time of harvest, rinse plates with PBS.
3) Add 3mL Accutase and incubate for 10-15 minutes at 37°C.
4) Remove cells to 15mL conical centrifuge tube and rinse dish with culture medium to collect residual cells.
5) Pellet cells at 300 x g for 5 minutes.
6) Wash pellet in PBS supplemented with cOmplete™, EDTA-free, Protease Inhibitor Cocktail Tablet (1 tablet per 50mL PBS).
7) Count number of cells and proceed to **SOP for cultured cells: nuclei, DNaseI treatment, crosslinking, and preserving cells for RNA** and **SOP: Cell purification using Percoll step gradients**.