

SOP: Propagation of HFF-Myc, Human Foreskin Fibroblast Cells Expressing Canine cMyc
Date modified: 12/14/2010
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Ordering Information

HFF-Myc Human Foreskin Fibroblast Cells Expressing Canine cMyc were received from Dr. Beverly Torok-Storb, Fred Hutchison Cancer Research Center, Seattle, WA.

Notes:

This is an adherent cell line. HFF-Myc are human foreskin fibroblasts containing a virally integrated vector expressing the canine cMyc gene (PLXL Myc ires NEO retro vector; Torok-Storb, unpublished). Growth and general cell behavior is similar to HFF human foreskin fibroblast cells.

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. Characterized Fetal Bovine Serum (HyClone, Cat# SH30071)
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 HFF-Myc

DMEM with 4.5g/L glucose, L-glutamine, and sodium pyruvate Medium
10% Characterized FBS
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, then dispense contents of ampoule into a T25 flask with 10mL of warm growth media.
- 4) Allow cells to recover overnight in 37°C, 5% CO₂ 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.
- 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) Perform 1:3 to 1:6 cell split as needed.
- 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.
- 2) Remove cells from flasks according to protocol described above under “Sub-culture”.
- 3) Examine viability using Trypan blue staining (SOP TP-7).