SOP: **Propagation of GM04503D**
Date modified: **11/14/2011**
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Ordering Information

GM04503D may be ordered from Coriell Cell Repositories. Proliferating cells are shipped in a T25 flask with 50-60mL of media.

To order starter cultures:
Name/Catalogue #: GM04503D

Notes:
This is an adherent fibroblast strain from a 31 year old female monozygotic twin to GM04504A.

Materials List

1. Eagle's MEM with 2mM L-glutamine and Earle's salts Medium (Cellgro, Cat# 10-010-CM)
2. Characterized Fetal Bovine Serum (HyClone, Cat# SH30071)
3. Non-essential Amino Acids, 100X solution (Invitrogen, Cat# 11140-050)
4. Penicillin-Streptomycin Solution (200X) (Cellgro, Cat# 30-001-CI)
5. T25, T75, T225 tissue culture flasks
6. Corning conical centrifuge tubes (15mL and 50mL)
7. Graduated pipets (1, 5, 10, 25, 50mL)
8. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
9. Accutase Enzyme Cell Detachment Medium (EBiosciences, Cat# 00-4555)
10. Freezing Medium (Growth medium containing 5% DMSO)
11. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
12. Cryovials (Nunc, Cat# 368632)
13. Cryo 1^{o}C Freezing Container (Nalgene Cat# 5100-0001)
14. Eppendorf Centrifuge 5810R
15. Revco UltimaII -80^{o}C Freezer
16. Thermolyne Locator 4 Liquid Nitrogen Freezer
17. Hemocytometer
18. Micropipet w/ P20 tips
19. Microscope

Growth Medium for GM04503D

Eagle's MEM with 2mM L-glutamine and Earle's salts Medium
15% Characterized FBS
Non-essential Amino Acids (1X)
Pen-Strep (1X)

Procedure

A. Receipt of Proliferating Cells

1) Swab down outside of flask with 70% ethanol.
2) Equilibrate unopened T25 flask overnight in 37^{o}C, 5% CO_2 humidified incubator to allow cells to recover.

B. Sub-culture

1) The next day after receipt, aspirate shipping medium and replace with fresh medium.
2) Propagate cells until density reaches 70-80% confluence.
3) Aspirate medium.
4) Wash cells with warm 1X PBS.
5) Add 10mL of Accutase and return to incubator for 10-15 minutes, or until cells detach.
6) Immediately remove cells, rinse flask with warm 1X PBS to collect residual cells, and pellet at 500 x g for 5 minutes (4^{o}C).
7) Gently re-suspend cell pellet in warm medium.
8) Perform 1:3 cell split as needed.
9) 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^{o}C in a Nalgene Cryo 1^{o}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).