

SOP: Propagation of GM04504A
Date modified: 11/14/2011
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Ordering Information

GM04504A 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 #: GM04504A

Notes:

This is an adherent fibroblast strain from a 31 year old female monozygotic twin to GM04503D.

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°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 GM04504A

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°C, 5% CO₂ 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°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°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).