**SOP:** **Propagation of AG04449**
**Date modified:** **12/14/09**
**Modified by:** **T. Canfield (UW)**

## Ordering Information

AG04449 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 #: AG04449 (Fetal Buttock/Thigh Fibroblast)

Notes:
This is a strain of dermal fibroblasts (adherent).

## Materials List

1. Eagle's MEM with Earle's salts and L-Glutamine (Cellgro Cat# 10-010-CM)
2. Characterized Fetal Bovine Serum (HyClone Cat# SH30071)
3. Non-essential Amino Acids, 100X (Invitrogen Cat# 11140-050)
4. T225 culture flasks
5. Graduated pipets (1, 5, 10, 25, 50mL)
6. Penicillin-Streptomycin Solution, 200X (Cellgro Cat# 30-001-CI)
7. Phosphate Buffered Saline (1X PBS) (prepared from 10X stock Cellgro, Cat# 46-013-CM by dilution with sterile deionized water)
8. Freezing medium (growth medium containing 6% DMSO)
9. DMSO, Hybri-Max (Sigma-Aldrich D2650)
10. Cryovials (Nunc Cat# 368632)
11. Accutase – Enzyme Cell Detachment Medium (EBioscience, Cat# 00-4555)
12. Hemocytometer
13. Micropipet w/ P20 tips
14. Microscope

## Growth Medium for AG04449

Eagle's MEM with Earle's salts and L-Glutamine
15% 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 10mLs 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 ($4^oC$).
7) Gently re-suspend cell pellet in warm medium.
8) Perform 1:4 cell split as needed. Record each subculture event as a passage

**C. Maintenance and Generation of Seed Stocks**

1) Change medium the day after seeding and every 2-3 days thereafter. Use ~50mL of 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, a portion of the flasks should be sub-cultured using Accutase as above under "Sub-culture" and the cell pellet resuspended in freezing medium.
3) Cells are dispensed into cryovials (2 million cells per 1mL aliquot) and frozen in a $-80^oC$ isopropanol cryo-freezing container overnight.
4) Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

**D. Harvest**

1) Do not use cells that have been passaged more than 8 times.
2) Remove cells from flasks according to protocol described above under 'Sub-culture'.
3) Examine viability using Trypan blue staining (SOP TP-7).