SOP: **Propagation of GM12873**
Date modified: **6/3/2009**
Modified by: **Jeff Goldy/M. Dorschner (UW)**

Ordering Information

GM12873 may be ordered from Coriell Cell Repositories. Proliferating cells are shipped in a T25 flask with 10-20ml of media.

To order starter cultures:
Name/Catalogue #: GM12873

Notes:
This cell line grows in suspension and should be maintained at a density between 2x10^5 cells/ml and 1x10^6cells/ml.

Materials List

1. RPMI 1640 with 2mM L-glutamine (cellgro Cat# 10-040-CM)
2. Fetal Bovine Serum (cellgro Cat# 35-016-CV)
3. T225 culture flasks
4. Graduated pipets (1, 5, 25, 50mL)
5. Penicillin-Streptomycin Solution, 100X (Cellgro, Cat#300-002CI)
6. Hemocytometer
7. Micropipet w/ P20 tips
8. Microscope
9. Freezing medium (growth medium containing 6% DMSO)

Growth Medium for GM06990

RPMI 1640 with 2mM L-glutamine
15% FBS
Pen-Strep (1X)

Procedure

A. Receipt of proliferating cells and generation of seed stocks

1) Equilibrate unopened T25 flask overnight in 37^{o}C, 5% CO_2 humidified incubator to allow cells to recover.
2) Cells should be counted the next day and split to achieve a cell density of 200,000-500,000 cells/ml.
3) Cells should be incubated in upright flasks with vented or loose caps.
4) Upon reaching the desired number, cells should be spun down, rinsed with 1X PBS, resuspended in freezing medium.
5) Cells are dispensed into cryovials (2 million per aliquot) and frozen in a -80^{o}C isopropanol bath overnight.
6) Cryovials are transferred the next day to liquid nitrogen for long term storage.

B. Sub-culture and Maintenance

1) Maintain culture at a cell density between 2x10^5 and 1x10^6 cells/ml.
2) Cells will either need to be fed again after 3-4 days or split depending on the cell density. Splitting can be performed by centrifuging cells at 500g for 5 minutes, decanting growth medium and rinsing in sterile 1X PBS. Cells should then be resuspended in fresh growth medium to achieve a density 2x10^5 and 1x10^6 cells/ml.

C. Harvest

1) Pass cells until the desired number of cells is reached.
2) Spin down and rinse cells as described above in Sub-culture and maintenance.