SOP: **Propagation of GM12864**
Date modified: **12/10/2009**
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Ordering Information

GM12864 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 #: GM12864 (Male B-Lymphocyte Utah Pedigree 1459 Repository Linkage Family)

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

Materials List

1. RPMI 1640 with 2mM L-glutamine (Cellgro, Cat# 10-040-CM)
2. Characterized Fetal Bovine Serum (HyClone, Cat# SH30071)
3. T225 culture flasks
4. Graduated pipets (1, 5, 10, 25, 50mL)
5. Penicillin-Streptomycin Solution, 200X (Cellgro, Cat#30-001-CI)
6. Phosphate Buffered Saline (1X PBS) (prepared from 10X stock Cellgro, Cat# 46-013-CM by dilution with sterile deionized water)
7. Freezing medium (growth medium containing 6% DMSO)
8. DMSO, Hybri-Max (Sigma-Aldrich Cat# D2650)
9. Cryovials (Nunc Cat# 368632)
10. Hemocytometer
11. Micropipet w/ P20 tips
12. Microscope

Growth Medium for GM12864

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 with a hemocytometer the next day and diluted to achieve a cell density of 2x10^5 cells/ml to 5x10^5 cells/ml.
3) Cells should be incubated in upright flasks with vented caps.
4) Upon reaching the desired number of cells, cells should be spun down at 500 X g (4^{o}C) for 5 minutes, cell pellet rinsed with 1X PBS, and resuspended in freezing medium.
5) Cells are dispensed into cryovials (2 million cells per 1mL aliquot) and frozen in a -80^{o}C isopropanol cryo-freezing container overnight.
6) Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

B. Sub-culture and Maintenance

1) Take cell counts with a hemocytometer every 48 hours to maintain the culture at a cell density between 2x10^5 and 1x10^6 cells/ml.
2) Add fresh warm medium when appropriate to maintain cell density and expand culture to desired number of cells. Splitting can be performed by centrifuging cells at 500 X g for 5 minutes (4^{o}C), aspirating spent growth medium, and rinsing cell pellet in sterile 1X PBS. Cells should then be resuspended in fresh growth medium to achieve a density 2x10^5 and 1x10^6 cells/ml.
3) Record each subculture event as a passage.

C. Harvest

1) Passage cells until the desired number of cells is reached.
2) Pellet cells and rinse with 1X PBS as described above in "Sub-culture and Maintenance".
3) Examine viability using Trypan blue staining (SOP TP-7).