

**SOP:** Propagation of GM12865  
**Date modified:** 12/10/2009  
**Modified by:** E. Giste/T. Canfield (UW)

### **Ordering Information**

GM12865 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 #: GM12865 (Female 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  $2 \times 10^5$  cells/ml and  $1 \times 10^6$  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, 100X (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 GM12865**

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  $37^\circ\text{C}$ , 5%  $\text{CO}_2$  humidified incubator to allow cells to recover.
- 2) Cells should be counted the next day with a hemocytometer and diluted to achieve a cell density of  $2 \times 10^5$  cells/ml to  $5 \times 10^5$  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  $500 \times g$  ( $4^\circ\text{C}$ ) 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  $-80^\circ\text{C}$  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  $2 \times 10^5$  and  $1 \times 10^6$  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  $500 \times g$  ( $4^\circ\text{C}$ ) for 5 minutes, 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  $2 \times 10^5$  and  $1 \times 10^6$  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).