

**SOP:** Propagation of GM12869  
**Date modified:** 7/21/2011  
**Modified by:** R.S. Hansen/T. Canfield (UW)

### **Ordering Information**

GM12869 cells are ordered from Coriell Cell Repositories. Proliferating cells are shipped in a T25 flask with 10–20mL of media.

To order starter cultures:

Name/Catalog #: GM12869 (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# SH-30071-03)
3. T225 culture flasks
4. Graduated serological pipets (1, 5, 10, 25, 50mL)
5. Penicillin-Streptomycin Solution, 200X (Cellgro, Cat# 30-001-CI)
6. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
7. Freezing medium (growth medium containing 6% DMSO)
8. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
9. Cryovials (Nunc, Cat# 368632)
10. Cryo 1°C Freezing Container (Nalgene, Cat# 5100-0001)
11. Eppendorf Centrifuge 5810R
12. Revco UltimaII -80°C Freezer
13. Thermolyne Locator 4 Liquid Nitrogen Freezer
14. Hemocytometer
15. Micropipet w/ P20 tips
16. Microscope

### **Growth Medium for GM12869**

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

### **Procedure**

#### **A. Receipt of Proliferating Cells and Generation of Seed Stocks**

- 1) Equilibrate unopened T25 flask overnight in 37°C, 5% CO<sub>2</sub> 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 between  $2 \times 10^5$  cells/mL and  $5 \times 10^5$  cells/mL.

- 3) Cells should be incubated in upright flasks with vented caps.
- 4) Upon reaching the desired number of cells, the cells should be pelleted at 500 x g (4°C) for 5 minutes, the 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°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 x g for 5 minutes (4°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 between  $2 \times 10^5$  and  $5 \times 10^5$  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).