

**SOP:** Propagation of M059J, Human Brain Malignant Glioblastoma Cells  
**Date modified:** 11/14/2011  
**Modified by:** T. Canfield (UW)

### **Ordering Information**

Human Brain Malignant Glioblastoma Cells M059J can be ordered from ATCC as a frozen ampoule with  $1.5 \times 10^6$  cells per 1mL volume. This is an adherent cell line.

Name: M059J—Human Brain Malignant Glioblastoma Cells  
ATCC #: CRL-2366

### **Materials List**

1. 1:1 mixture of DMEM and Ham's F12 Media with 2.5mM L-glutamine, 15mM HEPES, 0.5mM Sodium Pyruvate, 1.2g/L Sodium Bicarbonate (ATCC, Cat# 30-2006)
2. Characterized Fetal Bovine Serum (HyClone, Cat# SH30071)
3. Non-essential Amino Acids, 100X (Invitrogen, Cat# 11140-050)
4. Penicillin-Streptomycin Solution (200X) (Cellgro, Cat# 30-001-CI)
5. T25, T75, T225 tissue culture flasks
6. Corning conical centrifuge tubes (15mL and 50mL)
7. Graduated pipets (1, 5, 10, 25, 50mL)
8. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
9. Accutase Enzyme Cell Detachment Medium (EBiosciences, Cat# 00-4555)
10. Freezing Medium (Growth medium containing 5% DMSO)
11. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
12. Cryovials (Nunc, Cat# 368632)
13. Cryo 1°C Freezing Container (Nalgene Cat# 5100-0001)
14. Eppendorf Centrifuge 5810R
15. Revco UltimaII -80°C Freezer
16. Thermolyne Locator 4 Liquid Nitrogen Freezer
17. Hemocytometer
18. Micropipet w/ P20 tips
19. Microscope

### **Growth Medium for M059J**

1:1 mixture of DMEM and Ham's F12 Media with 2.5mM L-glutamine, 15mM HEPES, 0.5mM Sodium Pyruvate, 1.2g/L Sodium Bicarbonate  
10% Characterized FBS  
Non-essential Amino Acids (0.5X = 0.05mM)  
Pen-Strep (1X)

### **Procedure**

#### **A. Receipt of Frozen Cells and Starting Cell Culture**

- 1) Immediately place frozen cells in liquid nitrogen storage until ready to culture.
- 2) When ready to start cell culture, quickly thaw ampoule in a 37°C water bath.

- 3) As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol, then dispense contents of ampoule into a 15mL Corning centrifuge tube containing 9mL complete culture medium.
- 4) Pellet cells at 125 x g for 7 minutes (4°C).
- 5) Resuspend cell pellet in 10mL complete culture medium and dispense into a T25 flask.
- 6) To incubate the culture, place the flask in a 37°C, 5% CO<sub>2</sub> humidified incubator.

#### **B. Sub-culture**

- 1) Propagate cells until density reaches 70-80% confluence.
- 2) Aspirate medium.
- 3) Wash cells with warm 1X PBS.
- 4) Add 15mL of Accutase and return to incubator for 10-15 minutes, or until cells detach.
- 5) Immediately remove cells, rinse flask with warm 1X PBS to collect residual cells, and pellet at 500 x g for 5 minutes (4°C).
- 6) Gently re-suspend cell pellet in warm medium.
- 7) Perform 1:6 to 1:8 cell split as needed.
- 8) Record each subculture event as a passage.

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

- 1) Change media the day after seeding and every 2-3 days thereafter. Use 50mL of growth 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, the major portion of the flasks should be sub-cultured using Accutase as above under “Sub-culture” and a small portion should be set aside as a seed stock. The cell pellet for the seed stock should be resuspended in freezing medium.
- 3) Cells in freezing medium are dispensed into cryovials (2 million cells per 1 mL aliquot) and frozen at -80°C in a Nalgene Cryo 1°C freezing container overnight.
- 4) Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

#### **D. Harvest**

- 1) Passage cells until the desired number of cells is reached.
- 2) Remove cells from flasks according to protocol described above under “Sub-culture”.
- 3) Examine viability using Trypan blue staining (SOP TP-7).