

**SOP:** Propagation of CMK (Human Acute Megakaryocytic Leukemia Cells)  
**Date modified:** 11/12/09  
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### **Ordering Information**

CMK can be ordered from DSMZ (Braunschweig, Germany) as a frozen ampoule.

Name: CMK (Human Acute Megakaryocytic Leukemia Cells)  
DSMZ#: ACC 392

### **Notes:**

This cell line grows in suspension and should be maintained at a density between  $5 \times 10^5$  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. Sodium Pyruvate, 100X (Cellgro Cat# 25-000-CI)
4. T225 culture flasks
5. Graduated pipets (1, 5, 25, 50mL)
6. Penicillin-Streptomycin Solution, 200X (Cellgro, Cat# 30-001-CI)
7. Hemocytometer
8. Micropipet w/ P20 tips
9. Microscope

### **Growth Medium for CMK**

RPMI 1640 with 2mM L-glutamine  
10% FBS  
Sodium Pyruvate (1X)  
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) Quickly thaw ampoule in 37°C water bath.
- 3) Transfer thawed cells to a T25 flask with 10ml of warm growth media.
- 4) Allow cells to recover overnight in 37°C, 5% CO<sub>2</sub> humidified incubator.
- 5) The next morning, spin down cells at 500 X g (4°C) for 5 minutes, aspirating medium from cell pellet.
- 6) Re-suspend cells in warm fresh medium at a volume to yield a density of  $5 \times 10^5$  cells/mL.

#### **B. Sub-culture and Maintenance**

- 1) Take cell counts with a hemacytometer every 48 hours to maintain the culture at a cell density between  $5 \times 10^5$  and  $1 \times 10^6$  cells/mL (maximal density is at  $1 \times 10^6$ - $1.5 \times 10^6$  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 of  $5 \times 10^5$  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).