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 (Brauschweig, 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 5x10^5 cells/ml and 1x10^6cells/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^{o}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^{o}C, 5% CO_2 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 5x10^5 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 5x10^5 and 1x10^6 cells/mL (maximal density is at 1x10^6-1.5x10^6 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 5x10^5 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).