

SOP: Propagation of GM06990
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Ordering Information

GM06990 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 #: GM06990

Notes:

This cell line grows in suspension and should be maintained at a density between 2×10^5 cells/ml and 1×10^6 cells/ml.

Materials List

1. RPMI 1640 with 2mM L-glutamine (cellgro Cat# 10-040-CM)
2. Fetal Bovine Serum (cellgro Cat# 35-016-CV)
3. T225 culture flasks
4. Graduated pipets (1, 5, 25, 50mL)
5. Penicillin-Streptomycin Solution, 100X (Cellgro, Cat#300-002CI)
6. Hemocytometer
7. Micropipet w/ P20 tips
8. Microscope
9. Freezing medium (growth medium containing 6% DMSO)

Growth Medium for GM06990

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°C, 5% CO₂ humidified incubator to allow cells to recover.
- 2) Cells should be counted the next day and split to achieve a cell density of 200,000-500,000 cells/ml.
- 3) Cells should be incubated in upright flasks with vented or loose caps.
- 4) Upon reaching the desired number, cells should be spun down, rinsed with 1X PBS, resuspended in freezing medium.
- 5) Cells are dispensed into cryovials (2 million per aliquot) and frozen in a -80°C isopropanol bath overnight.
- 6) Cryovials are transferred the next day to liquid nitrogen for long term storage.

B. Sub-culture and Maintenance

- 1) Maintain culture at a cell density between 2×10^5 and 1×10^6 cells/ml.
- 2) Cells will either need to be fed again after 3-4 days or split depending on the cell density. Splitting can be performed by centrifuging cells at 500g for 5 minutes, decanting growth medium and rinsing in sterile 1X PBS. Cells should then be resuspended in fresh growth medium to achieve a density 2×10^5 and 1×10^6 cells/ml.

C. Harvest

- 1) Pass cells until the desired number of cells is reached.
- 2) Spin down and rinse cells as described above in Sub-culture and maintenance.