

SOP: Propagation of NB4
Date modified: 12/14/09
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Ordering Information

NB4 can be ordered from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) as a frozen ampoule.

Name: NB4 (Human Acute Promyelocytic Leukemia Cells)
Catalogue #: ACC-207

Notes:

This cell line grows in suspension and should be maintained at a density between 0.5×10^6 cells/ml and 1×10^6 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. T225 culture flasks
4. Graduated pipets (1, 5, 10, 25, 50mL)
5. Penicillin-Streptomycin Solution, 200X (Cellgro, Cat#30-001-CI)
6. Phosphate Buffered Saline (1X PBS) (prepared from 10X stock Cellgro, Cat# 46-013-CM by dilution with sterile deionized water)
7. Freezing medium (growth medium containing 6% DMSO)
8. DMSO, Hybri-Max (Sigma-Aldrich Cat# D2650)
9. Cryovials (Nunc Cat# 368632)
10. Hemocytometer
11. Micropipet w/ P20 tips
12. Microscope

Growth Medium for NB4

RPMI 1640 with 2mM L-glutamine
10% FBS
Pen-Strep (1X)

Procedure

A. Receipt of Frozen Cells, Starting Cell Cultures, and Generation of Seed Stocks

- 1) Immediately place frozen cells in liquid nitrogen freezer 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% CO₂ humidified incubator.
- 5) The next morning, spin down cells out of diluted shipping medium (which contains DMSO) at 500 X g (4°C) for 5 minutes, aspirating medium from cell pellet.
- 6) Re-suspend cells in warm fresh media at a volume to yield a density of 0.5×10^6 cells/ml.
- 7) Upon reaching the desired number of cells, cells should be spun down, rinsed with 1X PBS, and resuspended in freezing medium.
- 8) Cells are dispensed into cryovials (2 million cells per 1mL aliquot) and frozen in a -80°C isopropanol cryo-freezing container overnight.

9) 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 0.5×10^6 and 1×10^6 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 $500 \times g$ 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 0.5×10^6 and 1×10^6 cells/ml.
- 3) Record each subculture 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).