SOP: **Propagation of NB4**
Date modified: **7/29/2009**
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Ordering Information

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

Name: NB4
Catalogue #: ACC-207

Notes:
This cell line grows in suspension and should be maintained at a density between 0.5x10^6 cells/ml and 1x10^6cells/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 NB4

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

Procedure

A. Receipt of Frozen cells and starting cell cultures.

1) Immediately place frozen cells in liquid nitrogen storage incubator.
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 over night in 37^{o}C, 5% CO_2 humidified incubator.
5) The take cell count and spin down cells, 500g for 5 minutes, then decant old media
6) Re-suspend cells in warm fresh media at a volume to yield a density of 0.5x10^6 cells/ml.

B. Sub-culture and Maintenance

1) Maintain culture at a cell density between 0.5x10^6 and 1x10^6 cells/ml.
2) Cells will either need to be fed again after 2-3 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 0.5x10^6 and 1x10^6 cells/ml.

C. Harvest

1) Pass cells until the desired number of cells is reached. Cells can be harvested at a density of 1.5-2.0 x10^6 cells.
2) Spin down and rinse cells as described above in Sub-culture and maintenance.