SOP: **Propagation of Sk-N-SH**
Date modified: **9/5/2008**
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Ordering Information

SK-N-SH can be ordered from ATCC as a frozen ampule.

Name: Sk-N-SH, Neuroblastoma
ATCC #: HTB-11

Notes:
This is an adherent cell line. Cells are differentiated with retinoic acid (ATRA) for 48 hours prior to harvesting.

Materials List

1. RPMI 1640 with 2mM L-glutamine (Cellgro Cat# 10-040-CM)
2. Fetal Bovine Serum (Cellgro Cat# 35-016-CV)
3. Sodium Pyruvate (Cellgro Cat# 25-000-CI)
4. T225 culture flasks
5. *all trans*-Retinoic acid (Sigma, Cat #R2625)
6. Graduated pipets (1, 5, 25mL)
7. Penicillin-Streptomycin Solution, 100X (Cellgro, Cat#300-002CI)
8. Hemocytometer
9. Micropipet w/ P20 tips
10. Microscope

Growth Medium for SK-N-Sh

RPMI 1640 with 2mM L-glutamine
Sodium Pyruvate 10mM
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 T75 flask with 40ml of warm growth media.
4) Allow cells to recover over night in 37^{o}C, 5% CO_2 humidified incubator.
5) Pour off medium the next day, replace with fresh medium and return to incubator.

B. Sub-culture

1) Propagate cells until density reaches 70-80% confluence.
2) Decant medium.
3) Wash cells with warm 1X PBS.
4) Add 8mLs of Accutase and return to incubator for 10-15 minutes.
5) Immediately remove cells and pellet at 500 xg for 3 minutes (4^{o}C).
6) Wash cells 2X with 1X PBS.

7) Gently re-suspend cell pellet in warm medium.
8) Perform 1:3 to 1:8 cell split as needed.
9) Record each subculture event as a passage.

C. Maintenance

1) Change media the day after seeding and 1-2 times per week thereafter.
   Use ~50mLs of medium per T225 flask.

D. Differentiation

1) Upon reaching the desired cell number, add growth medium containing 6uM *all trans*-retinoic acid. Cells should be cultured for 48 hours in differentiation medium.

E. Harvest

1) Do not use cells that have been passed more than 8 times
2) Remove cells from flasks according to protocol described above under 'subculturing'
3) Examine viability using trypan blue staining (SOP)