SOP: Propagation of SK-N-SH (ATCC HTB-11)

ENCODE4 - Version 1

Information

Name: SK-N-SH
ATCC #: HTB-11
Organism*: Homo sapiens*, human
Tissue: brain; derived from metastatic site; bone marrow
Morphology: epithelial
Culture Properties: adherent
Biosafety Level: 1
Disease: neuroblastoma: 4-year-old female
Applications: Used as a target cell line kin cell mediated cytotoxicity assays and a suitable transfection host.
Population Doubling Time: about 22 hours [PDL = [log (number of the cells you have) - log (number of the cells you cultured)]/0.301]

Materials List

1. MEM (CORNING Cellgro; Cat # 10-10-CV or 10-10-CM)
2. Heat Inactivated Fetal Bovine Serum (CORNING; Cat # 35-016-CV)
3. Penicillin-Streptomycin 10,000U (Life Technologies, Cat # 15140 or Corning Cellgro, Cat # 300-002-CI)
4. Phosphate Buffered Saline (1X PBS) w/o Ca^{2+}, Mg^{2+} (CORNING Cellgro; Cat # 21-040-CM)
5. TrypLE Express (Life Technologies; Cat # 12604)
6. T75, T182 tissue culture treated flasks
7. Graduated pipets (2, 5, 10, 25, 50 ml)
8. Freezing medium (growth medium containing 95%; DMSO, 5%)
9. DMSO (Fisher; Cat # BP-231-100)
10. Cryovials (Sarstedt; Cat # 72-694-006)
11. TC20 cell counter (Bio-Rad)
12. Counting Slides (Bio-Rad; Cat # 145-0011)
13. 0.40% Trypan Blue Dye (Bio-Rad; Cat # 145-0013)
14. Microscope

Growth Medium for SK-N-SH

MEM
10% FBS
Pen-Strep (1X, 100U)

Procedure

A. Receipt of Frozen Cells and Starting Cell Culture

1) Immediately place frozen cells in liquid nitrogen freezer storage until ready to culture.
2) When ready to start cell culture, quickly thaw ampoule in a 37^{o}C water bath.
3) As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol, then dispense contents of ampoule into a T75 flask with 20 ml of warm growth media.
4) Allow cells to recover overnight in 37^{o}C, 5% CO2 humidified incubator.
5) The next morning, the diluted DMSO-containing shipping/cryopreservation medium is aspirated from the cell layer and replaced with fresh medium.

B. Sub-culture

Volumes used in this protocol are for 75 cm^2 and/or 182 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of the other size.

1) Propagate cells until density reaches 70-80% confluence.
2) Remove and discard culture medium.
3) Wash cells with room temperature (or warm) 1X PBS. (To remove all traces of serum that contains trypsin inhibitor.)
4) Add 3.0 ml (T-75) or 10.0 ml (T182) of TrypLE and return to incubator for 7-15 minutes, or until cells detach.

Note: To avoid clumping DO NOT agitate the cells by hitting or shaking the flask while waiting for the cells to detach.

5) Add 3.0-5.0 ml (T-75) or 10.0-13.0 ml (T182) of complete growth medium and aspirate the cells by gentle pipetting.
6) Transfer the cell suspension to the centrifuge tube and centrifuge at approximately 1,300 rpm for 4 minutes. Discard the supernatant.

7) Gently re-suspend the cell pellet in fresh growth medium. Perform 1:3 to 1:8 cell split as needed.
8) Record each subculture event as a passage. (Preferably don't use cells for more than 8 passages after thawing.)

C. Maintenance and Generation of Seed Stocks

1) Change media the every 3-4 days. Use 25 ml of growth medium per T75 flask, or 50 ml per T182 flask.
2) Following first or second passage after receipt of cells and with sufficient number of cells to continue maintenance and expansion, the major portion of the flasks should be sub-cultured using TrypLE as above under "Sub-culture" and a small portion should be set aside as a seed stock. The cell pellet for the seed stock should be re-suspended in freezing medium.
3) Cells in freezing medium are dispensed into cryovials (1-5 million cells per 1 ml aliquot) and frozen in a -80^{o}C cryo-freezing container overnight.
4) Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

D. Harvest

1) Detach cells from flasks as described above under "Sub-culture".
2) Examine viability using Trypan blue staining and count cells. Transfer desired amount of cells to new tube and centrifuge and wash cells with PBS once for your application if needed.