Cell Growth Protocol for SK-N-MC Cell Line

From: HudsonAlpha/Caltech ENCODE group
Date: 8/27/08
Prepared by: Norma Neff and Tim Reddy

SK-N-MC (ATCC number HB-10) cell culture and formaldehyde cross-linking

SK-N-MC is a neuroepithelioma cell line derived from a metastatic supra-orbital human brain tumor. The cells are adherent and epithelial-like in culture. The karyotype is pseudodiploid female with a modal chromosome number of 46. There are numerous chromosome abnormalities and marker chromosomes.

Cell culture protocol:

Growth medium: DMEM (Gibco/Invitrogen) + 10% fetal bovine serum (Hyclone) + 100 units/ml penicillin +100 μg/ml streptomycin + 5% CO_2 at 37^{o}C.

Liquid Nitrogen Storage: Complete growth medium supplemented with 5% (v/v) DMSO in 1-ml aliquots of approximately 5 x 10^6 cells.

1. Thaw 1ml aliquot of cells as quickly as possible in water bath at 37^{o}C. Transfer cells to 9mls of warm media in 15-ml conical tube. Mix gently. Spin at 1,200 rpm for 5 minutes to pellet cells. Discard media and resuspend pellet gently in 10 ml warm medium. Divide cells into two T-25 flasks containing 5 ml warm media. Place in incubator. After two days, remove the medium and add fresh media.

2. When cells are 70-90% confluent, split them 1:5. Remove and discard culture medium. Briefly rinse the cell layer with equal volume PBS pH 7.4 (Gibco/Invitrogen) and discard. Add 3 ml 0.25% (w/v) Trypsin + 0.53 mM EDTA (Gibco/Invitrogen) solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

Note: To avoid clumping, do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Trypsin solution warmed to 37°C can be used to facilitate dispersal. Add 7 ml complete growth medium and collect cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels. If the cells stay as large visible clumps and do not disperse well, then collect the cells after Trypsin treatment into a conical tube and centrifuge at 1,000 rpm for 5 minutes. Aspirate off media and resuspend the cells in 7-10 ml fresh media before diluting into new culture dishes.

Cell cross-linking and harvest:

3. Cells are plated into 150-mm plates for cross-linking and harvest (30-35 ml per dish). One or two plates are trypsinized and counted. Save these cells for DNA or other types of analysis. Plates harvested at 70-90% confluence contain 5-9 x 10^6 cells.

4. Add formaldehyde to 1% directly to the cells on plates. Swirl to mix. After 10 minutes at room temperature glycine is added to 0.125 M, swirl to mix and leave at room temperature for 5 minutes. Pour off medium and wash with 30 ml cold PBS pH 7.4.

5. Add 8 ml cold Farnham Lysis buffer (5 mM PIPES pH 8.0 / 85 mM KCl / 0.5% NP-40) + Roche Protease Inhibitor Cocktail Tablet (Complete 11836145001; for 50 ml, add protease inhibitor tablet just before use) and scrap cells into 15-ml conical tubes. Spin at 1,000 rpm for 5 minutes. Remove supernatant and freeze cell pellets on dry ice. Store at -80^{o}C.