Cell Growth Protocol for U-87 cell line

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U-87 (ATCC number HTB-14) cell culture and formaldehyde cross-linking

U-87 is a glioblastoma, astrocytoma cell line derived from human malignant gliomas. The cells are adherent epithelial cells. The karyotype is hypodiploid female with a modal chromosome number of 44 in 48% of cells and a 5.9% rate of higher ploidy. There are several marker chromosomes.

Cell culture protocol:

Growth medium: MEM (Gibco/Invitrogen) + 10% fetal bovine serum (HyClone) + 100 units/ml penicillin +100 µg/ml streptomycin + 5% CO_2 at 37°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 1 ml aliquot of cells as quickly as possible in water bath at 37°C. Transfer cells to 9 ml warm media in 15-ml conical tube. Mix gently. Centrifuge 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 1:4. Remove and discard culture medium. Briefly rinse the cell layer with an 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 minutes). Add 7-10 ml complete growth medium and collect cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels. Warming the trypsin solution to 37°C can expedite cell release. 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 the media and resuspend the cells in 7-10 ml fresh media before diluting into new culture dishes.

Cell cross-linking and harvest:

3. Plate cells into 150-mm plates for cross-linking and harvest (30-35 ml per dish). Trypsinize and count one or two plates. Save these cells for DNA or other types of analysis. Harvest plates at 70-90% confluence; these contain 9.6×10^6 - 1.3×10^7 cells.

4. Add formaldehyde to 1% directly to the cells on plates. Swirl to mix. After 10 minutes at room temperature, add glycine 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 scrape cells into 15-ml conical tubes. Centrifuge at 1,000 rpm for 5 minutes. Remove supernatant and freeze cell pellets on dry ice. Store at -80°C.