Cell Growth Protocol for A549 Cell Line

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A549 (ATCC number CCL-185) cell culture and formaldehyde cross-linking

A549 is a human epithelial cell line derived from a lung carcinoma tissue. The cells are adherent in culture. The karyotype is hypotriploid male with a modal chromosome number of 12. There are numerous chromosome abnormalities and marker chromosomes.

Cell culture protocol:

Growth medium: F12/K (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 a 1-ml aliquot of cells as quickly as possible in water bath at 37^{o}C. Transfer cells to 9 ml 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 between 1:4 and 1:12. To do so, remove and discard culture medium. Add 0.25% (w/v) Trypsin + 0.53 mM EDTA (Gibco/Invitrogen) solution at 37°C to barely coat cells and observe cells under an inverted microscope until cell layer is dispersed (usually within 5-15 minutes). Add 3x to 5x complete growth medium and collect cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels.

Cell cross-linking and harvest:

3. Plate cells into 150-mm plates for cross-linking and harvest (50 ml per dish). Trypsinize and count one or two plates. Save these cells for DNA or other types of analysis. Plates harvested at 70-90% confluence contain 1-2 x 10^7 cells.

4. To induce GR, add dexmethasone to 100 nM or ethanol vehicle control to each plate (10 μl/50-ml plate), and return cells to incubator.

5. After 1 hour, 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 50 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 50-ml conical tubes (4 plates per tube). Spin at 1,000 rpm for 5 minutes. Remove supernatant and freeze cell pellets on dry ice. Store at -80^{o}C.