Cell Growth Protocol for Jurkat Cell Line

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Jurkat (ATCC number TIB-152) cell culture and formaldehyde cross-linking

Jurkat Clone E6-1 is a human T lymphoblastoid cell line derived from an acute T cell leukemia. The cells are suspension lymphoblasts. The karyotype is pseudodiploid human male cell line. The modal chromosome number is 46, occurring in 74% with polyploidy at 5.3%.

Cell culture protocol:

Growth medium: Advanced RPMI 1640 (Gibco/Invitrogen) + 10% fetal bovine serum (Hyclone) + 10 mM Hepes + 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 1ml 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 a 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 media and add fresh media.

2. When cells are 6-8x10^5 cells/ml, split them 1:4 with fresh media. Add appropriate aliquots of the cell suspension to new culture vessels (T25 = 10 ml; T75 = 50 ml; T150 = 100 ml maximum volume). Grow cells to no more than 8 x 10^5 cells/ml. Disperse clumps gently for counting.

Large-scale cell growth (2-4 liters), cross-linking and harvest:

3. Grow cells in T150 flasks to a concentration of 5-8 x 10^5 cells/ml. Divide cells into four pools. Save aliquots of cells (1 x 10^6 to 5 x 10^7) from each pool for DNA or other types of analysis.

4. Fix cells as recommended for K562 cells. Concentrate cells by centrifugation at 1,200 rpm for 5 minutes at room temperature and resuspend in RPMI with no additives at 2 x 10^7 cells/ml. RPMI + 2% formaldehyde (freshly made) is added in equal volume to cells. Mix on rotator for 10 minutes at room temperature. Add glycine to 0.125 M, mix on a

rotator at room temperature for 5 minutes. Centrifuge at 800 rpm at room temperature for 10 minutes. Remove supernatant. Resuspend cells in cold PBS, pH 7.4. Transfer 10^8 cells to 15-ml conical tubes. Centrifuge at 800 rpm for 5 minutes at 4^oC. Aspirate off PBS and freeze pellets on dry ice. Store at -80^oC.