Cell Culture Protocol for HCT 116 cells

HCT 116 (ATCC number CCL-247): colorectal carcinoma cell line

Growth medium: McCoy's 5a Medium (GIBCO # 16600) + 10% FBS + 100 units/ml penicillin + 100 μg/ml streptomycin (GIBCO # 15140-122).

Protocol:

1. Take out the HCT 116 stock vial from liquid nitrogen (we freeze at $5x10^6$ cells per vial) and thaw it in 37° water bath. Suspend thawed cells in 5 ml growth media.

2. Centrifuge at 1500 rpm for 5 min, discard media.

3. Resuspend cell pellet in 15ml growth media and transfer cells into a 75 sq. cm. flask. Cells are grown in a 37ºC incubator at 5% CO2.

4. Split cultures when they reach 70-90% confluency.
   Briefly rinse the cell layer with PBS (Gibco/Invitrogen). Add 5 ml 0.25% (w/v) Trypsin + 0.53 mM EDTA (Gibco/Invitrogen) solution to the flask of attached cells and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 minutes). Add 5 ml complete growth medium and collect cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels. We typically split 1:5 (adding about $5x10^6$ cells per 75 sq. cm flask). Incubate cultures at 37°C. Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

5. If $5x10^6$ cells are plated onto a 75 sq. cm flask, the culture typically reaches 70-90% confluency in 2-3 days and is ready to split or harvest for experiments.

6. Cells can be stored as a stock in liquid nitrogen at 5 million cells/ml in straight serum (FBS) containing 10% DMSO.