SOP: Propagation of HCT116

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Name: HCT116
Tissue Source: large intestine; colon
Species: *Homo sapiens*, human
Sex of cell: male
Culture Properties: adherent

Information: HCT116 is an adherent cell line isolated from the colon of a patient with colon cancer. It has a mutation in codon 13 of the ras-proto-oncogene. This cell line is near-diploid and has a relatively stable genetic profile, making it a valuable in vitro model. This line can be utilized in cancer research and gastrointestinal (GI) research.

Medium for HCT116

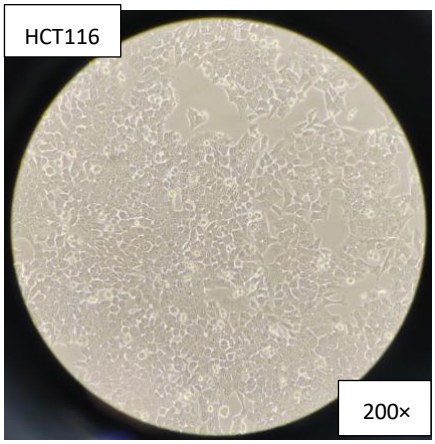
1. Growth medium: 89% Gibco™ BASIC DMEM, High Glucose (Gibco, Cat. no. C11965500BT) + 10% FBS (ExCell Bio, Cat. no. FSP500) + 1% Gibco™ Penicillin-Streptomycin (Gibco, Cat. no. 15140122).
2. Freezing medium: 90% FBS + 10% DMSO.

Culture conditions

1. Stilling culture: for example--in T-175 cm^2 flask with 20 ml medium upright position.
2. 37°C under 5% CO_2.

Initiate HCT116 cell culture from frozen stock and passage cells

1. **Initiate:** Pre-warm growth medium at 37℃ at least 1 h before use.
2. Thaw the cells (1 ml, ~1*10^7 cells/tube) in 37℃ water bath (~2 min). Decontaminate the outside of the vial with 75% ethanol.
3. Transfer the cells immediately into a 15-ml tube containing 5 mL growth medium.
4. Centrifuge the 15-ml tube at 1000 rpm for 5 minutes.
5. Aseptically decant the medium containing DMSO and resuspend the cell pellet in 2 ml of pre-warmed fresh growth medium and transfer to a T-175 flask with 18 ml pre-warmed fresh growth medium.
6. Place the flask in a 37℃ incubator with 5% CO_2 for 16-24 h.
7. Remove the cell medium and add 20 ml fresh growth medium.
8. Check cells morphology and confluence under microscope.
9. After recovery, they will need to be passaged every 2-3 days. Once cells confluence is approximately 90%, they should be passaged.
10. **Passage:** Remove the supernatant, add 10 ml DPBS to wash the adherent cells.
11. Add 2.5 ml trypsin to digest the adherent cells for 2-3 minutes at 37°C.
12. Add 5.5 ml fresh culture medium to neutralize the trypsin, mix well, and transfer the cell suspension into the 15-ml tube.
13. Centrifuge at 1000 rpm for 5 minutes at RT.
14. Discard the supernatant, resuspend the cell pellet in fresh pre-warmed growth medium, and split the cells at a 1:5-1:8 ratio.



Freeze HCT116 cells

1. Before starting, label 5 cryovials and prepare 5 ml of Freezing medium: 4.5 ml of FBS + 0.5 ml of DMSO (90% FBS +10% DMSO).
2. Transfer trypsin digested cells of T-175 (~5*10^7 cells) into a 15-ml tube.
3. Spin the tube down at 1000 rpm for 5 min at RT.
4. Discard the medium and resuspend the cells with 5 ml of Freezing medium.
5. Re-suspend the cells with 5 ml of Freezing medium (~1*10^7 cells/ml).
6. Aliquot 1 ml of the cell suspension per vial.
7. Freeze cells in a control rate freezer to –80°C.
8. Transfer container to –80°C and hold for 24 hours to allow for a slow freezing process.
9. Transfer vials to liquid nitrogen for long-term storage.

References

https://www.atcc.org/products/ccl-247