

**SOP:** Propagation of HCT116, Human Colorectal Carcinoma Cells (ATCC)  
**Date modified:** 03/29/2010  
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### **Ordering Information**

Human Colorectal Carcinoma Cells (HCT-116) can be ordered from ATCC as a frozen ampoule. This is an adherent cell line.

Name: HCT-116 –Human Colorectal Carcinoma Cells  
ATCC #: CCL-247

### **Materials List**

1. McCoy's 5A Modified Medium (ATCC Cat# 30-2007)
2. Characterized Fetal Bovine Serum (HyClone Cat# SH30071)
3. T25, T75, T225 culture flasks
4. Graduated pipets (1, 5, 10, 25, 50mL)
5. Penicillin-Streptomycin Solution, 200X (Cellgro, Cat# 30-001-CI)
6. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
7. Accutase Enzyme Cell Detachment Medium (EBiosciences, Cat# 00-4555)
8. Freezing medium (growth medium containing 6% DMSO)
9. DMSO, Hybri-Max (Sigma-Aldrich Cat# D2650)
10. Cryovials (Nunc Cat# 368632)
11. Hemocytometer
12. Micropipet w/ P20 tips
13. Microscope

### **Growth Medium for HCT116**

McCoy's 5A Modified Medium  
10% FBS  
Pen-Strep (1X)

### **Procedure**

#### **A. Receipt of Frozen Cells and Starting Cell Culture**

- 1) Immediately place frozen cells in liquid nitrogen freezer storage until ready to culture.
- 2) When ready to start cell culture, quickly thaw ampoule in a 37°C water bath.
- 3) As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol, then dispense contents of ampoule into a T25 flask with 10ml of warm growth media.
- 4) Allow cells to recover overnight in 37°C, 5% CO<sub>2</sub> humidified incubator.
- 5) The next morning, the diluted DMSO-containing shipping/cryopreservation medium is aspirated from the cell layer and replaced with fresh medium.

#### **B. Sub-culture**

- 1) Propagate cells until density reaches 70-80% confluence.
- 2) Aspirate medium.
- 3) Wash cells with warm 1X PBS.
- 4) Add 15mL of Accutase and return to incubator for 10-15 minutes, or until cells detach.
- 5) Immediately remove cells, rinse flask with warm 1X PBS to collect residual cells, and pellet at 500 x g for 5 minutes (4°C).
- 6) Gently re-suspend cell pellet in warm medium.
- 7) Perform 1:3 to 1:8 cell split as needed.

8) Record each subculture event as a passage.

**C. Maintenance and Generation of Seed Stocks**

- 1) Change medium the day after seeding and every 2-3 days thereafter. Use 50mL of growth medium per T225 flask.
- 2) Following first or second passage after receipt of cells and with sufficient number of cells to continue maintenance and expansion, the major portion of the flasks should be sub-cultured using Accutase as above under “Sub-culture” and a small portion should be set aside as a seed stock. The cell pellet for the seed stock should be resuspended in freezing medium.
- 3) Cells in freezing medium are dispensed into cryovials (2 million cells per 1mL aliquot) and frozen in a -80°C isopropanol cryo-freezing container overnight.
- 4) Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

**D. Harvest**

- 1) Passage cells until the desired number of cells is reached.
- 2) Remove cells from flasks as described above under “Sub-culture”.
- 3) Examine viability using Trypan blue staining (SOP TP-7).