**SOP:** **Propagation of HT-29 Colorectal Adenocarcinoma Cells**
**Date modified:** **8/18/2014**
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## Ordering Information

The HT-29 cell line was isolated from a primary colon tumor of an adult female.
HT-29 cells can be ordered from ATCC as a frozen ampoule.

Name: HT-29, colorectal adenocarcinoma
ATCC #: HTB-38

Notes:
This is an adherent cell line.

## Materials List

1. Dulbecco's Modified Eagle's Medium without Sodium Pyruvate (Corning Cellgro Cat#10-017-CV)
2. Sodium Pyruvate (Corning Cellgro Cat#25-000-CI)
3. Fetal Bovine Serum (Corning Cellgro Cat# 35-016-CV)
4. T75 & T225 culture flasks
5. Sarstedt Graduated Serological Pipets (1, 5, 10, 25, 50mL)
6. Corning Conical Centrifuge Tubes (15mL and 50mL)
7. BD Falcon 2mL Aspirating Pipets
8. Penicillin-Streptomycin Solution (100X) (Corning Cellgro Cat# 30-002-CI)
9. Phosphate Buffered Saline (1X PBS) (Corning/Cellgro, Cat# 21-040-CM)
10. Accutase Enzyme Cell Detachment Medium (Innovative Cell Technologies, Cat# AT 104)
11. Freezing Medium (Growth medium containing 10% DMSO)
12. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
13. Cryovials (Nunc, Cat# 368632)
14. Cryo 1°C Freezing Container (Nalgene Cat# 5100-0001)
15. Eppendorf Centrifuge 5810R
16. Revco UltimaII -80°C Freezer
17. Thermolyne Locator 4 Liquid Nitrogen Freezer
18. Hemocytometer
19. Micropipet w/ P20 Tips
20. Microscope

## Growth Medium for HT-29

Dulbecco's Modified Eagle's Medium without Sodium Pyruvate
Sodium Pyruvate
10% FBS
Pen-Strep (1X)

## Procedure

### A. Receipt of frozen cells and starting cell cultures.

1) Immediately place frozen cells in liquid nitrogen storage incubator.
2) Quickly thaw ampoule in 37°C water bath.
3) Transfer thawed cells to a T75 flask with 20mL of warm growth media.
4) Allow cells to recover overnight in 37°C, 5% $CO_2$ humidified incubator.
5) Remove medium the next day, replace with fresh medium and return to incubator.

### B. Subculture

1) Propagate cells until density reaches 70-80% confluence.
2) Decant medium.
3) Wash cells with warm 1X PBS.
4) Add 10mL of Accutase and return to incubator for 10-15 minutes.
5) Immediately remove cells and pellet at 500 x g for 5 minutes (4°C)
6) Gently re-suspend cell pellet in warm medium.
7) Perform 1:4 cell split as needed.
8) Record each subculture event as a passage.

### C. Maintenance and generation of seed stocks

1) Change media the day after seeding and every 2-3 days thereafter, using ~50mL of medium per T225 flask.
2) To prepare frozen stocks, cells in freezing medium are dispensed into cryovials (2 million cells per 1mL aliquot) and frozen at -80°C in a Nalgene Cryo 1°C freezing container overnight.
3) Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

### D. Harvest

1) Remove cells from flasks according to protocol described above under 'subculture'.
2) Examine viability using trypan blue staining.