SOP: Propagation of HeLa S3 (ATCC CCL-2.2) Using Spinner Flask

Information

Name: HeLa S3
ATCC#: CCL2.2
Tissue: cervix
Product Format: frozen
Morphology: epithelial
Culture Properties: adherent
Biosafety Level: 2 (cells contain human papilloma virus)
Disease: adenocarcinoma; 31-year-old Black female

ENCODE Number: HO2075
Lot Number: 4490244

Materials List

1. DMEM (Life Technologies; Cat#11965)
2. Calf Serum (Hyclone; Cat #SH30072)
3. Penicillin-Streptomycin 10,000U/mL (Life Technologies; Cat#15140))
4. Phosphate Buffered Saline (1X PBS) w/o Ca^{2+}, Mg^{2+} (CORNING Cellgro; Cat# 21-040-CM)
5. S-MEM (Life Technologies; Cat#11380)
6. Alpha MEM (Life Technologies; Cat#12571)
7. TrypLE Express (Life Technologies; Cat#12604)
8. 150 mm Tissue Dishes, T75 culture flasks
9. 500 mL Spinner Flask (Wheaton Science Products; Cat#356882)
10. 1000 mL Spinner Flask (Wheaton Science Products; Cat#356884)
11. Graduated pipets (1, 5, 10, 25, 50 mL)
12. Freezing medium (growth medium containing 95%; DMSO, 5%)
13. DMSO (Fisher; Cat#BP-231-100)
14. Cryovials (Sarstedt; Cat #72-694-006)
15. Wheaton Micro-Stir (Wheaton Service Products; Cat W900701-A)
16. TC20 cell counter (Bio-Rad)
17. Counting Slides (Bio-Rad; Cat# 145-0011)
18. Microscope

Growth Medium for HeLa S3

DMEM
5% FCS
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^{o}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 150 mm tissue dish with 20 ml of warm growth media.
4) Allow cells to recover overnight in 37^{o}C, 5% CO2 humidified incubator.
5) The next morning, the diluted DMSO-containing shipping/cryopreservation medium is aspirated from the cell layer and replaced with fresh medium.

Note: We thaw the cells onto a dish because we have found that the initial growth of cells is better this way.

B. Sub-culture

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of the other size.

1) Propagate cells until density reaches 60-70% confluence.
2) Aspirate medium.
3) Wash cells with room temperature (or warm) 1XPBS.
4) Add 5 mL (tissue dish), or 3 mL (T-75) of TrypLE and return to incubator for 3 minutes, or until cells detach.
5) Add 5 mL (tissue dish), or 7 mL of complete medium and aspirate the cells by gentle pipetting.
7) Perform 1:4 to 1:10 cell split as needed.
8) Incubate cultures at 37^{o}C, 5% CO2 humidified incubator.
9) Change Medium 2 to 3 times per week.
10) Record each subculture event as a passage.

C. Generation of Seed Stocks

1) Change media the day after seeding and every 3-4 days thereafter. Use 25-30 mL of growth medium per T75 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 TrypLE 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 (1-2 million cells per 1mL aliquot) and frozen in a -80^{o}C cryo-freezing container overnight.
4) Cryovials are transferred the next day to liquid nitrogen freezer for long-term storage.

D. Spinner Culture

Initial Spinner Medium: [1 part of S-MEM + 1 part of Alpha MEM] + 5% calf serum + 1X Pen/Strep

Expansion Spinner Medium; [4 parts of S-MEM + 1 part of Alpha MEM] + 5% calf serum + 1X Pen/Strep

1) Passage cells until the desired number of cells is reached.
2) Trypsinize cells, resuspend in Initial Spinner Medium, and count.
3) Add Initial Spinner Medium to the trypsinized cells so that cells are at a density of 1-2 X 10^5 cells/mL and transfer to 500 mL spinner flask containing 200 mL medium (this results in a spinner culture of cells at initial density of 1-2 X 10^5/mL. Spin at slow-medium setting, sufficient to maintain good aeration, but slow enough to avoid damaging the cells.
2) Expand cells when they reach 5 X 10^5 cells/mL by transferring into larger spinner flasks, using Expansion Spinner Medium. When expanding, dilute to 1-2 X 10^5/mL and harvest when cells reach 5 X 10^5 cells/mL

Note:

1) The Initial Spinner Medium has a higher ratio of Alpha MEM than the Expansion Spinner Medium because the higher calcium in the Alpha MEM is helpful when initially establishing the cells in spinner, but can lead to problems with clumping in the larger flasks.

2) Spinner flasks should not be filled more than half way to maintain sufficient aeration; as cell number increase, larger sized flasks will have to be used. Side arm caps must be kept loose to allow for air exchange. Check carefully for contamination-it is often hard to distinguish this from cell debris, which accumulates as cells maintained in spinner culture. After use, treat the emptied flasks with a 10% bleach solution for 30 minutes and then thoroughly rinse with distilled water. Add a small amount of dH_2O to flasks before autoclaving to avoid breakage during autoclaving-decant prior to use.