SOP: Propagation of MCF7 (ATCC HTB-22)

Information

Name: MCF7
ATCC#: HTB-22
Tissue: mammary gland/breast; derived from metastatic site: pleural effusion
Product Format: frozen
Morphology: epithelial
Culture Properties: adherent
Biosafety Level: 1
Disease: adenocarcinoma: 69-year-old Caucasian female
Population Doubling Time: 29 hours

Materials List

1. MEM with 2mM L-glutamine and Earle's salts (Fisher; Cat# 10-010-CM)
2. Heat Inactivated Fetal Bovine Serum (Life Technologies; Cat# 10082147)
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. TrypLE Express (Life Technologies; Cat#12604)
6. T75, T525 culture flasks
7. Graduated pipets (1, 5, 10, 25, 50 mL)
8. Freezing medium (growth medium containing 95%; DMSO, 5%)
9. DMSO (Fisher; Cat#BP-231-100)
10. Cryovials (Sarstedt; Cat #72-694-006)
11. TC20 cell counter (Bio-Rad)
12. Counting Slides (Bio-Rad; Cat# 145-0011)
13. Microscope

Growth Medium for MCF7

MEM with 2mM L-glutamine and Earle's salts
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^{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 a T75 flask with 20 ml of warm growth media.
4) Allow cells to recover overnight in 37^{o}C, 5% CO_2 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

Volumes used in this protocol are for 75 cm^2 and/or 525 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of the other size.

Note: If floating cells are present, it is recommended that they be transferred at the first two (2) subcultures as described below

1) Propagate cells until density reaches 70-80% confluence.
2) Remove culture medium to a centrifuge tube.
3) Wash cells with room temperature (or warm) 1XPBS.
4) Add 3 mL (T-75) or 30 mL (T525) of TrypLE and return to incubator for 3 minutes, or until cells detach.
5) Add 7 ml (T-75) or 30 mL (T525) of complete medium and aspirate the cells by gentle pipetting.
6) Transfer the cell suspension to the centrifuge tube with medium and cells from step 2, and centrifuge at approximately 1,500 rpm for 5 minutes. Discard the supernatant. Resuspend the cell pellet in fresh growth medium.
7) Perform 1:3 to 1:6 cell split as needed.
8) Incubate cultures at 37^oC, 5% CO_2 humidified incubator.
9) Change Medium 2 to 3 times per week.
10) Record each subculture event as a passage.

It is not necessary to transfer floating cells of subsequent. Skip step 2, and 6.

C. Maintenance and Generation of Seed Stocks

1) Change media the day after seeding and every 3-4 days thereafter. Use 100 mL of growth medium per T525 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^oC 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