SOP: **Propagation of MCF7**
Date modified: **12/14/09**
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Ordering Information

MCF7 can be ordered from ATCC as a frozen ampoule.

Name: MCF7, mammary gland adenocarcinoma
ATCC #: HTB-22

Notes:
This is an adherent cell line.

Materials List

1. Eagle's MEM with Earle's salts and L-Glutamine (Cellgro Cat# 10-010-CM)
2. Characterized Fetal Bovine Serum (HyClone Cat# SH30071)
3. Non-essential Amino Acids, 100X (Invitrogen, Cat#11140-050)
4. Insulin from bovine pancreas, cell-culture tested, 200X (Sigma-Aldrich Cat# I6634, prepared in acidified water per manufacturer's specifications)
5. T75 and T225 culture flasks
6. Graduated pipets (1, 5, 10, 25, 50mL)
7. Penicillin-Streptomycin Solution, 200X (Cellgro, Cat# 30-001-CI)
8. Phosphate Buffered Saline (1X PBS) (prepared from 10X stock Cellgro, Cat# 46-013-CM by dilution with sterile deionized water)
9. Accutase Enzyme Cell Detachment Medium (EBioscience, Cat# 00-455)
10. Freezing medium (growth medium containing 6% DMSO)
11. DMSO, Hybri-Max (Sigma-Aldrich Cat# D2650)
12. Cryovials (Nunc Cat# 368632)
13. Hemocytometer
14. Micropipet w/ P20 tips
15. Microscope

Growth Medium for MCF7

Eagle's MEM with Earle's salts and L-Glutamine
Non-essential Amino Acids (1X)
0.01 mg/ml Bovine Insulin
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) Quickly thaw ampoule in 37^{o}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^{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

1) Propagate cells until density reaches 70-80% confluence.
2) Aspirate medium.
3) Wash cells with warm 1X PBS.
4) Add 15mLs 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^oC).
6) Gently re-suspend cell pellet in warm medium.
7) Perform 1:3 to 1:6 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 ~50mLs of 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, a portion of the flasks should be sub-cultured using Accutase as above under "Sub-culture" and the cell pellet resuspended in freezing medium.

3) Cells are dispensed into cryovials (2 million cells per 1 mL aliquot) and frozen in a -80^oC 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) Pellet cells and rinse with 1X PBS as described above in "Sub-culture and Maintenance".
3) Examine viability using Trypan blue staining (SOP TP-7).