SOP: **Propagation and Hormone Treatment of MCF7, Human Mammary Gland Adenocarcinoma Cells**
Date modified: **01/31/2012**
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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 positive for both estrogen and progesterone receptors.
The hormone treatment procedure is similar to that published by Zwart et al., 2011 (EMBO J. 30:4764–4776).

Materials List

1. DMEM, high glucose, no glutamine, no phenol red (Invitrogen, Cat# 31053-028)
2. DMEM, high glucose, no glutamine (Invitrogen, Cat# 11960-044)
3. Fetal Bovine Serum (Sigma-Aldrich, Cat# F0926)
4. Charcoal Stripped Serum (Invitrogen, Cat# 12676)
5. L-Glutamine, 100X Solution (Invitrogen, Cat# 25030)
6. Non-essential Amino Acids, 100X Solution (Invitrogen, Cat# 11140-050)
7. Sodium Pyruvate, 100X Solution (Invitrogen, Cat# 111360)
8. Penicillin-Streptomycin Solution, 100X (Invitrogen, Cat# 15140)
9. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
10. Trypsin-EDTA (Invitrogen, Cat# 25200)
11. Insulin from bovine pancreas, cell-culture tested, 200X (Sigma-Aldrich Cat# I6634, prepared in acidified water per manufacturer's specifications)
12. Beta-Estradiol (Sigma-Aldrich Cat# E2758); reconstituted to a stock concentration of 10^{-4} M in ethanol (1000X)
13. Ethanol, 200 proof (Decon Laboratories Inc., Cat# 2716)
14. T75, T225 tissue culture flasks
15. 150mm tissue culture dishes
16. Corning conical centrifuge tubes (15mL and 50mL)
17. Graduated serological pipets (1, 5, 10, 25, 50mL)
18. Freezing Medium (growth medium containing 10% DMSO)
19. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
20. CryoVials (Nunc, Cat# 368632)
21. Cryo 1°C Freezing Container (Nalgene, Cat# 5100-0001)
22. Eppendorf Centrifuge 5810R
23. Revco UltimaII -80°C Freezer
24. Thermolyne Locator 4 Liquid Nitrogen Freezer
25. Hemocytometer
26. Micropipet w/ P20 tips
27. Microscope

Growth Medium for Human MCF7 Cells

DMEM, high glucose, no glutamine
10% FBS
L-Glutamine (1X)
Non-essential Amino Acids (1X)
Sodium Pyruvate (1X)

Pen-Strep (1X)
0.01 mg/ml Bovine Insulin

Charcoal Stripped Medium for Hormone Treatment of Human MCF7 Cells

DMEM, high glucose, no glutamine, no phenol red
10% Charcoal Stripped Serum
L-Glutamine (1X)
Non-essential Amino Acids (1X)
Sodium Pyruvate (1X)
Pen-Strep (1X)
0.01 mg/ml Bovine Insulin

Procedure

A. Receipt of Frozen Cells and Starting Cell Cultures

1. Immediately place frozen cells in liquid nitrogen storage until ready to culture.
2. When ready to start cell culture, quickly thaw ampoule in 37^{o}C water bath until ice crystals disappear.
3. Swab outside surface of the ampoule with 70% ethanol and then dispense contents of ampoule into a T75 tissue culture flask with 20mL of warm growth medium.
4. Allow cells to recover overnight in a 37°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. Human MCF7 cells can be grown to confluency without affecting subsequent passaging. These cells grow in clumps or foci and do not grow in even monolayers.
2. Aspirate medium.
3. Wash cell layer with warm 1X PBS.
4. Add 5mL of Trypsin-EDTA and leave at room temperature for 5 minutes, or until cells detach.
5. Immediately remove detached cells to a centrifuge tube, rinse flask with warm 1X PBS to collect residual cells, and pellet at 500 x g for 5 minutes (4°C). Alternatively, trypsinized cells can be passaged directly by adding to fresh medium (no centrifugation necessary).
6. Gently resuspend cell pellet in warm growth medium.
7. Perform a 1:4 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 at -80°C in a Nalgene Cryo 1°C 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 at confluency.
2. Remove cells from flasks as described above under “Sub-culture.”
3. Examine viability using Trypan blue staining (SOP TP-7).

E. Hormone Treatment of Cells

1. Cells are split into 150mm tissue culture dishes at a concentration of 2 million cells per dish in phenol red-free, charcoal stripped medium.
2. Cells are maintained in phenol red-free, charcoal stripped medium for 2 days prior to hormone treatment.
3. Hormone treatments with estradiol are for 1 hour at a final concentration of 100nM.