SOP: **Propagation of IMR90, Normal Human Lung Fibroblasts (ATCC)**
Date modified: **03/29/2010**
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Ordering Information

Normal Human Lung Fibroblasts (IMR90) can be ordered from ATCC as a frozen ampoule. This is an adherent cell strain.

Name:	IMR90 – Normal Human Lung Fibroblasts
ATCC #:	CCL-186

Materials List

1. MEM with 2mM L-glutamine and Earle's salts (Cellgro Cat# 10-010-CM)
2. Characterized Fetal Bovine Serum (HyClone Cat# SH30071)
3. Non-essential Amino Acids, 100X solution (Invitrogen Cat# 11140-050)
4. Sodium Pyruvate, 100mM (Cellgro Cat# 25-000-CI)
5. T75, 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) (Cellgro, Cat# 21-040-CM)
9. Accutase Enzyme Cell Detachment Medium (EBiosciences, Cat# 00-4555)
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 IMR90

MEM with 2mM L-glutamine and Earle's salts
10% FBS
Non-essential Amino Acids (1X)
Sodium Pyruvate (1 mM)
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 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 15mL 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^{o}C).
6) Gently re-suspend cell pellet in warm medium.
7) Perform 1:2 to 1:8 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 in a -80^{o}C 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) Remove cells from flasks as described above under "Sub-culture".
3) Examine viability using Trypan blue staining (SOP TP-7).