SOP: **Propagation of HS5 Human Marrow Stromal Cells**
Date modified: **12/6/2010**
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Ordering Information

HS5 Human Marrow Stromal Cells were received from Dr. Beverly Torok-Storb, Fred Hutchison Cancer Research Center, Seattle, WA. They are fibroblastoid cells immortalized with HPV16 E6/E7 genes as described in Roecklein and Torok-Storb, 1995 *Blood* 85:997-1005; insert DNA is LXSN-16 E6E7 packaged in PA31.

Materials List

1. RPMI-1640 Medium (1X) (Invitrogen, Cat# 11875-093)
2. Fetal Bovine Serum, Certified, Heat-Inactivated (Invitrogen, Cat# 10082-147)
3. Penicillin-Streptomycin Solution (10,000 units Penicillin: 10,000µg Streptomycin) (100%) (Invitrogen, Cat# 15140-122)
4. Sodium Pyruvate Solution, 100mM (100X) (HyClone, Cat# SH30239.01)
5. L-Glutamine, 200mM, liquid (50X) (Invitrogen, Cat# 25030-149)
6. T75, T225 tissue culture flasks
7. Corning conical centrifuge tubes (15mL and 50mL)
8. Graduated pipets (1, 5, 10, 25, 50mL)
9. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
10. Accutase Enzyme Cell Detachment Medium (EBiosciences, Cat# 00-4555)
11. DMSO, ACS Spectrophotometric Grade (Sigma-Aldrich, Cat# 154938-100mL)
12. Cryovials (Nunc, Cat# 368632)
13. Cryo 1°C Freezing Container (Nalgene Cat# 5100-0001)
14. Eppendorf Centrifuge 5810R
15. Revco UltimaII -80°C Freezer
16. Thermolyne Locator 4 Liquid Nitrogen Freezer
17. Hemocytometer
18. Micropipet w/ P20 tips
19. Microscope

Growth Medium for HS5

RPMI-1640 Medium
10% Certified, Heat-inactivated FBS
Sodium Pyruvate (1mM)
Pen-Strep (1%)

Procedure

A. Receipt of Frozen Cells and Starting Cell Culture

1) Immediately place frozen cells in liquid nitrogen storage until ready to culture.
2) When ready to start cell culture, quickly thaw ampoule in a 37°C water bath.
3) As soon as ice crystals disappear, swab outside surface of the ampoule with 70% ethanol.

4) Place the contents of 2mL ampoule (1mL thawed cell suspension) into a 50mL Corning conical centrifuge tube and slowly add the following volumes of growth medium over 3 minutes per addition until the final concentration of DMSO is decreased to 1% (15mL total growth medium for 1mL cells; elapsed time: 18 minutes).
   a. 0.5mL 3 minutes
   b. 1.0mL 3 minutes
   c. 1.5mL 3 minutes
   d. 2.0mL 3 minutes
   e. 5.0mL 3 minutes
   f. 5.0mL 3 minutes
5) Incubate the tube on ice for 30 minutes.
6) Pellet cells at 300 x g for 5 minutes (4°C) and remove the supernatant.
7) Wash the cell pellet with 20mL growth medium; respin as in step 6.
8) Resuspend pellet in warmed growth medium and seed 1 million cells per T75 tissue culture flask.

B. Sub-culture

1) Propagate cells until density reaches 70-80% confluence (a confluent T75 flask contains 5-6 million cells).
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°C).
6) Gently re-suspend cell pellet in warm medium.
7) Perform 1:10 cell split as needed.
8) Record each subculture event as a passage.

C. Maintenance and Generation of Seed Stocks

1) Change media the day after seeding and every 2-3 days thereafter. Use 15mL of growth medium per T75 flask and 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.
3) The cell pellet for the seed stock should be resuspended in freezing solution (40% fetal bovine serum in RPMI-1640 medium supplemented with 1% Pen-Strep and 4mM glutamine) at a concentration of 4-10 million cells/ml and aliquot 0.5mL into a 2 ml cryovial (pre-cool cryovials and freezing solutions on ice to avoid toxicity by DMSO).
4) Add to the aliquoted cells an equal volume (0.5mL) of freezing solution plus 20% DMSO (final DMSO concentration is 10%).
5) Freeze cells at -80°C in a Nalgene Cryo 1°C freezing container overnight.
6) 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 according to protocol described above under “Sub-culture”.
3) Examine viability using Trypan blue staining (SOP TP-7).