

SOP: Propagation of HS27a Human Marrow Stromal Cells
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Modified by: T. Canfield (UW)

Ordering Information

HS27a Human Marrow Stromal Cells were received from Dr. Beverly Torok-Storb, Fred Hutchinson 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 HS27a

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).