SOP: **Propagation of MSC Human Primary Marrow Stromal Cells**
Date modified: **7/10/12**
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Ordering Information

Human primary marrow stromal cells were received from Dr. Beverly Torok-Storb, Fred Hutchison Cancer Research Center, Seattle, WA. These cells are primary fibroblastoid cells obtained from human bone marrow of normal donors as described in Roecklein and Torok-Storb, 1995 *Blood* 85:997-1005.

Notes:
These are adherent cells.

Materials List

1. Lineage Cell Depletion Kit (Miltenyi Biotec, Cat# 130-092-211)
2. MSC Basal Medium, 1X (ALLCELLS, Cat# MSC-002)
3. MSC Stimulatory Supplements (ALLCELLS, Cat# MSC-003)
4. RPMI-1640 Medium (1X) (Invitrogen, Cat# 11875-093)
5. Fetal Bovine Serum (FBS), Certified, Heat-Inactivated (Invitrogen, Cat# 10082-147)
6. Penicillin-Streptomycin Solution (10,000 units Penicillin: 10,000µg Streptomycin) (100%) (Invitrogen, Cat# 15140-122)
7. Sodium Pyruvate Solution, 100mM (100X) (HyClone, Cat# SH30239.01)
8. L-Glutamine, 200mM, liquid (50X) (Invitrogen, Cat# 25030-149)
9. T225 tissue culture flasks
10. Corning conical centrifuge tubes (15mL and 50mL)
11. Graduated pipets (1, 5, 10, 25, 50mL)
12. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
13. Accutase Enzyme Cell Detachment Medium (EBiosciences, Cat# 00-4555)
14. DMSO, ACS Spectrophotometric Grade (Sigma-Aldrich, Cat# 154938-100mL)
15. MACS Separator
16. Cryovials (Nunc, Cat# 368632)
17. Cryo 1°C Freezing Container (Nalgene, Cat# 5100-0001)
18. Eppendorf Centrifuge 5810R
19. Revco UltimaII -80°C Freezer
20. Thermolyne Locator 4 Liquid Nitrogen Freezer
21. Hemocytometer
22. Micropipet w/ P20 tips
23. Microscope

Growth Medium for MSC Human Primary Marrow Stromal Cells

MSC Growth Medium: Mixture of Media A and B (1:1)

Medium A:
MSC Basal Medium, 1X
MSC Stimulatory Supplements
Pen-Strep (1%)

Medium B:
RPMI-1640 Medium
10% Certified, Heat-Inactivated FBS
Sodium Pyruvate (1mM)

Procedure

A. Starting cell culture

Note: Marrow mononuclear cells were used to establish relatively short-term, primary cultures of marrow stromal cells using Lineage Cell Depletion Kit and magnetic separation according to the manufacturer's protocol (Miltenyi). The cells were first cultured in Medium A according to the manufacturer's protocol (ALLCELLS).

1. The marrow stromal cells are seeded into T225 tissue culture flasks with MSC Growth Medium and placed in a 37°C incubator with 5% CO_2 (0.7-2.0 x 10^6 cells/flask).

B. Sub-culture

1. Propagate cells until density reaches 70-80% confluence.
2. Aspirate medium.
3. Wash cells with room temperature 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 room temperature 1X PBS to collect residual cells, and pellet at 500 x g for 5 minutes (4°C).
6. Gently resuspend cell pellet in prewarmed medium.
7. Perform 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 the first or second passage of cells, 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% FBS in RPMI-1640 medium supplemented with 1% Pen-Strep and 4mM L-glutamine) at a concentration of 4-10 million cells/mL. Aliquot 0.5mL into a 2mL 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).