

SOP: Propagation of Normal Human BJ Skin Fibroblasts (BJ, ATCC)
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Ordering Information

BJ human skin fibroblasts derived from normal foreskin can be ordered from ATCC as a frozen ampoule with about 1×10^6 cells per 1mL volume. This is an adherent cell line.

Name: BJ
ATCC #: CRL-2522

Materials List

1. MEM with 2mM L-glutamine and Earle's salts Medium (Cellgro, Cat# 10-010-CM)
2. Characterized Fetal Bovine Serum (HyClone, Cat# SH-30071-03)
3. Sodium Bicarbonate, 7.5% (w/v) solution (Cellgro, Cat# 25-035-CI)
4. Sodium Pyruvate, 100mM (Cellgro, Cat# 25-000-CI)
5. Non-essential Amino Acids, 100X solution (Invitrogen, Cat# 11140-050)
6. Penicillin-Streptomycin Solution (200X) (Cellgro, Cat# 30-001-CI)
7. T25, T75, T225 tissue culture flasks
8. Corning conical centrifuge tubes (15mL and 50mL)
9. Graduated pipets (1, 5, 10, 25, 50mL)
10. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
11. Accutase Enzyme Cell Detachment Medium (EBiosciences, Cat# 00-4555)
12. Freezing Medium (Growth medium containing 5% DMSO)
13. DMSO, Hybri-Max (Sigma-Aldrich, Cat# D2650)
14. Cryovials (Nunc, Cat# 368632)
15. Cryo 1°C Freezing Container (Nalgene, Cat# 5100-0001)
16. Eppendorf Centrifuge 5810R
17. Revco UltimaII -80°C Freezer
18. Thermolyne Locator 4 Liquid Nitrogen Freezer
19. Hemocytometer
20. Micropipet w/ P20 tips
21. Microscope

Growth Medium for BJ

MEM with 2mM L-glutamine and Earle's salts Medium
10% Characterized FBS
Sodium Pyruvate (1mM)
Non-essential Amino Acids (1X)
Sodium Bicarbonate 1.5g/L
Pen-Strep (1X)

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, then dispense contents of ampoule into a T25 flask with 10mL of warm growth media.
- 4) Allow cells to recover overnight in 37°C, 5% CO₂ 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°C).
- 6) Gently re-suspend cell pellet in warm medium.
- 7) Perform 1:2 to 1:9 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 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 1 mL 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.
- 2) Remove cells from flasks according to protocol described above under “Sub-culture”.
- 3) Examine viability using Trypan blue staining (SOP TP-7).