

SOP: Propagation of BJ
Date modified: 7/29/2009
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Ordering Information

BJ-tert can be ordered from ATCC as a frozen ampoule.

Name: BJ, skin fibroblast
ATCC #: CRL-2522

Notes:

This is an adherent cell line.

Materials List

1. MEM with 2mM L-glutamine (Cellgro Cat# 10-010-CM).
2. Fetal Bovine Serum (Cellgro Cat# 35-016-CV)
3. Sodium Bicarbonate (Cellgro Cat# 25-035-CI)
4. Sodium pyruvate (Cellgro Cat# 25-000-CI)
5. Non-essential amino acids (Cellgro Cat# 25-025-CI)
6. T75 & T225 culture flasks
7. Graduated pipets (1, 5, 25mL)
8. Penicillin-Streptomycin Solution (100X) (Cellgro Cat# 30-002-CI)
9. Hemocytometer
10. Micropipet w/ P20 tips
11. Microscope

Growth Medium for BJ

MEM with 2mM L-glutamine
Non-essential amino acids
Sodium Pyruvate 1mM
10% FBS
Pen-Strep (1X)
Sodium Bicarbonate 1.5g/L

Procedure

A. Receipt of frozen cells and starting cell cultures.

- 1) Immediately place frozen cells in liquid nitrogen storage incubator.
- 2) Quickly thaw ampoule in 37°C water bath
- 3) Transfer thawed cells to a T75 flask with 40mLs of warm growth media.
- 4) Allow cells to recover over night in 37°C, 5% CO₂ humidified incubator.
- 5) Pour off medium the next day, replace with fresh medium and return to incubator.

B. Sub-culture

- 1) Propagate cells until density reaches 70-80% confluence.
- 2) Decant medium.
- 3) Wash cells with warm 1X PBS.
- 4) Add 8mLs of Accutase and return to incubator for 10-15 minutes.

- 5) Immediately remove cells and pellet at 500 xg for 5 minutes (4°C).
- 6) Wash cells 2X with 1X PBS.
- 7) Gently re-suspend cell pellet in warm medium.
- 8) Perform 1:2 to 1:9 cell split as needed.
- 9) Record each subculture event as a passage.

C. Maintenance

- 1) Change media the day after seeding and every 2-3 days thereafter.
Use ~50ml of medium per T225 flasks.

D. Harvest

- 1) Do not use cells that have been passed more than 8 times.
- 2) Remove cells from flasks according to protocol described above under 'subculturing'.
- 3) Examine viability using trypan blue staining (SOP).