

Cell Culture SOP: Propagation of hTERT-immortalized BJ fibroblasts.

Ordering Information

BJ-tert can be ordered from ATCC as a frozen ampoule.
Name: BJ, skin fibroblast . ATCC #: CRL-2522.

Description

This line is immortalized by expression of telomerase (hTERT).

Notes:

These are adherent cell lines.

Materials List

1. Knockout DMEM (Gibco Cat# 10829).
2. Fetal Bovine Serum (Atlanta Biologicals Cat# s11150).
3. Medium 199 (Gibco Cat# 11150).
4. L-Glutamine (Gibco Cat# 25030).
5. T75 & T225 culture flasks.
6. Graduated pipets (1, 5, 25mL).
7. Penicillin-Streptomycin Solution (100X) (Gibco Cat# 15140).
8. Hemocytometer.
9. Micropipet w/ P20 tips.
10. Microscope.

Growth Medium for BJ

Knockout DMEM.
14.5% FBS.
16.5% Medium 199.
1.76mM L-Glutamine.
0.88% Pen-Strep.

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 37oC water bath.
- 3) Transfer thawed cells to a T75 flask with 40mLs of warm growth media.
- 4) Allow cells to recover over night in 37oC, 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 trypsin and return to incubator for about 5 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).