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

Source of cells

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Reference- Hahn WC. Nature, 1999 Jul 29;400(6743):464-8.

Description

The line is immortalized by expression of telomerase (hTERT), also expresses both large and small T antigens of Simian virus 40 and H-RasV12.

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 (4oC).
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).