

Freeze Cell Lines in 2.0mL Cryogenic tubes

1. Trypsinize cells.
2. Resuspend in 10mls es cell media.
3. Place in 15 ml Falcon tube and spin for 5 min.
4. Suck off supernatant
5. Resuspend pellet in 5 mls PBS.
6. Homogenize then put 10u onto hemacytometer.
7. Count and average 2 grids
8. Multiply average number of cells by 1×10^4 .
9. Suck off PBS.
10. Resuspend cells in COLD freezing media (10% DMSO, 90% ES cell media) such that you have 1×10^7 cells/ml. Keep cells chilled while working.
11. Place 1 ml of 1×10^7 cells/ml in cryogenic tubes labeled with CL# and name and put into cryogenic freezing container. (The freezing container should be at RT and have fresh isopropanol every 5 uses).
12. Place in -80 .
13. Next day, place in -140 .
14. Be sure to enter relevant info into DB.

Ex. Ave 166 cells/grid x 10 (diluted) x $10^4 = 1.66 \times 10^7$. So resuspend in 5 ml total vol of freezing media and freeze 5 tubes containing 1 ml each.