

Freezing Down Mammalian Cells for Long-Term Storage

Be extra careful of sterile technique. Everything should be done in the hood.

1. Get your 2 ml screw-top tubes ready. Label top with cell line name, side with cell line name, date, and your initials.
2. Make freezing media:
For each tube you will need 800 ul of your complete media + 100 ul of FBS (final 20% FBS in DMEM). Generally one 10 cm plate can be frozen into 2 tubes)
3. Trypsinize cells, and put them all in a 50 ml conical tube to spin down 5 min. 1000 rpm
4. While spinning, put 100 ul of DMSO into each tube (10% DMSO final concentration).
5. Add appropriate amount of freezing media (1.8 mL per 10 cm plage)and resuspend cells.
6. Put 900 ul into each tube containing the 100 uL DMSO.
7. Place into Styrofoam box and put into -80 degree overnight
8. Put into liquid nitrogen storage container the next day.