How to Thaw Frozen Cells

1. Prepare cell growth medium. Refer to preferred protocol for media preparation.
2. Make sure to log cells taken out from the liquid nitrogen documentation system.

   NOTE: Make sure to preheat water bath to 37° C.

3. Properly label 50 mL conical tube with cell line designation and the label from the cryovial.
4. Remove frozen vial of cells from the liquid nitrogen dewar.
   NOTE: Thaw out immediately; DMSO, a component of freezing Solution II is toxic to cells.

5. Sample thawing should be conducted with gentle swirling of sample until all visible ice has melted.

   Approximate thaw time for a 1 ml sample in a cryovial is 3 minutes.

   NOTE: Swirling prevents formation of intracellular ice.

6. **DO NOT** allow sample to warm to 37°C. Cryovials should be cool to the touch when removed from bath. *Passive thaw is not recommended.*

   NOTE: Make sure to wear safety glasses, gloves and a face shield.

7. Spray vial with Ethanol (70%) before transferring to biosafety cabinet to prevent contamination.

8. Gently resuspend cells 2-3 times and transfer into 50 mL conical tube.
   NOTE: Make sure to perform all steps under a biosafety cabinet.

9. Slowly and drop-wise add 9 mL of cold growth medium (refer to respective protocol for each cell type being thawed) to cells while gently shaking conical tube in order to reduce osmotic swelling upon rehydration and improve post-freeze viability. For every 1 mL of cells add 9 mL of growth medium.

10. Centrifuge to pellet cells at 150g for 5 min at 4^{o}C.

11. Aspirate supernatant carefully without disturbing pellet.

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12. Re-suspend cells in 1-2 mL of growth medium. Make sure to have a homogeneous cell suspension by pipetting several times up and down.

13. Do a cell count to determine post-freeze viability.

14. Dilute cells with pre-warmed growth medium in culture vessel to appropriate cell plating density.

15. Make sure to write cell name, experimental condition, date, and passage number on culture vessel. Fill out a cell culture log for culturing cells.

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