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Thawing Frozen Cells

Outline
Thaw the cells rapidly, dilute them slowly, and reseed them at a high cell density. Incubate for 24 hr and change medium to remove remaining cryoprotectant.

Materials
Sterile

- Culture flask, T25
- Growth medium, with 10% Serum and 1% PenStrep
- Pipettes, 10 mL
- Pipettor tips, 1mL

Nonsterile

- Gloves
- Cryogloves and protective glasses
- Pipette Aid
- Pipettor, 100-1000 uL
- Sterile water at 37°C, 5 cm deep in a clean alcohol-swabbed container with lid
- Alcohol, 70% in spray bottle
- Kimwipes

Protocol

1. Prepare hood by swabbing with 70% alcohol
2. Check freezer logbook for the location of the ampoule to be thawed
3. Collect materials and reagents and place them in biosafety hood, swab with 70% alcohol
4. Prewarm medium to 37°C in water bath (~10 min before procedure), swab with 70% alcohol
5. Retrieve the ampoule from freezer (use cryogloves!), and check the label if it's the correct ampoule
6. In the hood, loosen the cap on the vial ¼ turn for 10 seconds to release any liquid nitrogen that may be trapped in the threads, then re-tighten the cap (OPTIONAL).
7. Place vial in a 37°C waterbath, hold and rotate the vial gently until contents are completely thawed
8. Blot ampoule dry, and swab with 70% alcohol
9. Place ampoule in biosafety hood
10. Transfer contents of ampoule to culture flask
11. Add medium slowly to the cell suspension: add 10-mL of medium over 2 min, first dropwise and then little faster, gradually diluting the cells and cryoprotectant
12. Replace cap on culture flask, and gently rock the flask to distribute cells evenly
13. Label the flask, initial and date
14. Place flask in an incubator, and incubate for 16-24 hr
15. After 16-24 hrs, inspect cells under inverted microscope: cells should be attached and look healthy
16. Replace medium with fresh medium, to remove remaining cryoprotectant and unattached dead cells
17. Return the flask to incubator
18. Clean hood by swabbing with 70% alcohol

SAFETY NOTES
If an ampoule was submerged in liquid nitrogen during storage it may have leaked and inspired liquid nitrogen. On thawing the ampoule may explode violently! You must wear protective goggles and a lab coat to protect yourself! A container with a lid must be used for thawing to contain any explosion.

Protective cryogloves should be used when retrieving ampoules from liquid nitrogen storage!