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Freezing Cells

Outline

Grow the culture to log phase, prepare a high concentration cell suspension (50× of normal seeding concentration) in freezing medium, aliquot into cryovials, and freeze slowly. Transfer vials to liquid nitrogen.

Materials

Sterile

- Culture to be frozen, in T150 culture flask
- Growth medium, with serum
- DMSO, dimethyl sulfoxide
- Nalgene cryogenic vials, 2mL (Cat. No. 5012-0020)
- D-PBS without calcium and magnesium (Cat. No. 21-031-CV, Mediatech)
- Trypsin, 0.25% with 2.21 mM EDTA in HBSS (Cat. No. 25-053-CI, Mediatech)
- Pipettes, 10 mL, 25 mL

Nonsterile

- Gloves
- Pipette Aid
- Alcohol, 70% in spray bottle
- Nalgene freezing container ("*Mr. Frosty*"), filled with isopropyl alcohol
- Cryogloves
- Freezing box
- Materials for cell counting (*see protocol for cell counting*)

Protocol

1. Prepare hood by swabbing with 70% alcohol
2. Collect materials and reagents and place them in biosafety hood, swab with 70% alcohol
3. Defrost Trypsin to 4°C in fridge, and prewarm medium and D-PBS in water bath to 37°C
4. Retrieve culture flask from the incubator
5. Examine cells on an inverted microscope: look for signs of cell deterioration or contamination
6. Check criteria for freezing: ~70% confluency and otherwise healthy looking cells
7. Prepare freezing medium: growth medium + 10% DMSO (6 mL for each T150 flask)
8. Label, initial and date cryogenic vials (6 vials for each T150 flask)
9. Place culture flask in biosafety hood
10. Remove the medium by aspirating
11. Rinse cells with 30 mL of D-PBS and trypsinize with 15 mL of trypsin (*see subculture protocol*)
12. Add 6 mL of freezing medium and disperse cells by repeated pipetting over the monolayer
13. Pipette the cell suspension up-and-down a few times to create a single-cell suspension
14. Withdraw about 100 μL of cell suspension into microfuge tube for cell counting (OPTIONAL)
15. Dispense 1 mL of cell suspension per vial, and cap the vials
16. Place vials in freezing container (Mr. Frosty), and place container in –80°C for 16-24 hr
17. Identify suitable location for the ampoules in liquid nitrogen storage tank
18. Pour a bit of liquid nitrogen into a freezing box, and place vials in there for transporting to LN_2 tank
19. Place freezing box in liquid nitrogen storage tank; this transfer must be done quickly (<2 min).

SAFETY NOTES

DMSO can penetrate many synthetic and natural membranes, including skin and rubber gloves. Potentially harmful substances can be carried into blood circulation through the skin and even through gloves! As such, DMSO should always be handles with caution, especially in the presence of toxic compounds.