How to Thaw Cryopreserved Cells

Thawing cryopreserved cells properly is crucial to ensure the viability and functionality of the cells. Using good technique and working quickly ensures that a high proportion of the cells survive the procedure. As with other procedures, HemaCare scientists recommend that you closely follow the steps provided to ensure best results.

Equipment

- Water bath at 37°C
- Biological Safety Cabinet (BSC)
- Liquid nitrogen freezer
- Serological and Micro-Pipettors
- Centrifuge

Notes

- For the best cell viability and recovery, please use DNase to prevent clumping caused by DNA leakage from any dead cells.
- Always wear personal protective equipment and use universal precaution when working with human-derived biological materials and liquid nitrogen.

Materials Needed

- Thawing media:
  - HBSS without calcium or magnesium
  - 10% heat inactivated Human AB Serum or Human Serum Albumin (HSA)
- Washing buffer:
  - PBS without calcium or magnesium
  - 0.5% Human Serum Albumin (HSA)
  - 2mM EDTA
- 70% ethanol
- Cryopreserved cells in cryovial
- Sterile conical tubes
- Disposable serological pipettes and micropipette tips

Protocol

1. Warm thawing media in 37°C water bath.
2. Obtain the cryovial containing the cryopreserved cells from liquid nitrogen storage. Quickly loosen cap slightly to relieve pressure within the tube, retighten, and place it into a 37°C water bath immediately.
3. Hold the cryovial in the water without submerging the cap area. Do not move the vial while thawing (no figure 8 motion or flicking). To prevent contamination, do not allow water to come in contact with the cryovial cap.
4. Remove the vial from water bath when sliver of ice remains. Clean the outside of the vial with 70% ethanol, and transfer the cryovial into a BSC.
5. Pipette 5mL of thawing media using 10mL serological pipette. With the same pipette, gently aspirate the cryopreserved cell solution from the cryovial into the media within the pipette. Let the cells diffuse with the thawing media.
6. Gently transfer the cryopreserved cells and thawing media in the pipette into a labeled conical tube.
7. Rinse the cryovial with an appropriate volume of thawing media.
8. Drop-wise, transfer the rinse to the conical tube containing the cells.
9. Slowly add thawing media to a total volume of 10 times of the volume of the cryovial.
10. Aseptically, take a sample for cell count and viability analysis.
11. Centrifuge the cell suspension at 400 x g for 10 minutes at room temperature (the centrifugation speed and duration can vary depending on the cell and conical tube types).
12. Check the clarity of the supernatant and visibility of a complete cell pellet, and transfer into BSC.
13. Aseptically aspirate the supernatant without disturbing the cell pellet.
14. Flick or tap the tube to loosen the cell pellet.
15. Re-suspend the cells in washing buffer/media gently.
16. Take sample for cell count and viability analysis.
17. *Optional: Centrifuge the cell suspension at 400 x g for 10 minutes at room temperature.
18. *Optional: Check the clarity of the supernatant and visibility of a complete cell pellet.
19. *Optional: Carefully remove the supernatant aseptically without disturbing the cell pellet.
20. Re-suspend the cells in desired media for further applications.

Tips and Tricks
1. Do not add Ca\(^{2+}\) or Mg\(^{2+}\) because these molecules activate the cells after thawing and this is stressful.
2. When transferring to cell wash, doing it dropwise is gentler. Agitating these already stressed cells is very hard on them. Also, dropwise transfer reduces introduction of air bubbles, which causes more agitation.
3. Always perform a count prior to further processing and after processing/resuspending the cell pellet.
4. For DNase, we recommend using Sigma or Invitrogen brand.

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