

How to Thaw Cells in a Cryopreserved Leukopak (Cryobag)

Thawing cryopreserved cells properly is crucial to the viability and functionality of the cells. Using good technique and working quickly ensures that a high proportion of the cells survive the procedure.

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 cryobag
- Sterile conical tubes
- Disposable serological pipettes and micropipette tips

Equipment

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

Protocol

1. Warm thawing media in 37°C water bath. Once warm, remove media from water bath, spray with 70% ethanol, and place in BSC.
2. Obtain the cryobag containing the cryopreserved cells from liquid nitrogen storage. Place it into a 37°C water bath immediately.
3. Hold the cryobag under the surface of the water without moving (**no figure 8 motion or flicking**).
4. Remove the cryobag from water bath when a sliver of ice remains. Clean the outside of the bag with 70% ethanol, and transfer into a BSC.
5. Cut port with sterile scissors and slowly transfer the thawed cells into a 50mL tube.

6. Dropwise, add an equal volume of thawing media to the cell suspension.
7. Rinse the bag with an appropriate volume of thawing media, and gently add to the conical tube.
8. Slowly add thawing media to a total volume of 2-5 times of the volume of the cryobag.
9. Aseptically, take a sample for cell count and viability analysis.
10. Centrifuge the cell suspension at 350– 400 x g for 10 minutes at room temperature (the centrifugation speed and duration can vary depending on the cell and conical tube types).
11. Check the clarity of the supernatant and visibility of a complete cell pellet, and transfer into BSC.
12. Aseptically aspirate the supernatant without disturbing the cell pellet.
13. Flick or tap the tube gently to loosen the cell pellet.
14. Re-suspend the cells in washing buffer or desired media for further application.
15. Take sample for cell count and viability analysis.

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.

Tips & 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.