

Technical Notes—How to Thaw Frozen Cells

Shipping and Storage

For short-term storage (< 1 month), store in -80° C freezer. For long-term storage (> 1 month), store in the “vapor phase” of a liquid nitrogen tank.

General Medium Requirements

- IMDM, MEM, or RPMI-1640 containing 10% FBS
- DNase I

Thawing Cells

1. In a 37°C water bath, warm medium (IMDM, MEM, or RPMI-1640) containing 10% FBS.
2. Clean the frozen vial with 70% alcohol before thawing. In a biosafety hood, twist the cap a quarter-turn to relieve pressure, and then retighten the cap.
3. In a 37°C water bath, quickly thaw the vial. Be careful not to submerge the entire vial in the water bath. Do not remove the vial until a tiny ice-crystal is left.
4. Remove vial from water bath and clean the outside of the vial with 70% alcohol.
5. In a biosafety hood, measure the cell suspension volume.
6. Count using the Trypan Blue Method. Take a 10µl sample from the vial. Mix 10µl of the sample with 10µl of trypan blue. Dilute the cells if necessary and count the number of cells on a hemacytometer to determine the viability.

N = # of cells counted on all 4 squares of a hemacytometer
 d = dilution factor

Equation for Cell Count: # of cells/ vial = $N / 4 \times 2 \times d \times \text{___} \text{ml}$
= $\text{___} \times 10^4$

Equation for Viability: # of cells excluded by trypan blue/ total number of cells $\times 100\%$ = $\text{___}\%$

7. (Optional) In order to avoid occasional clumping, when thawing units with large cell numbers (> 100x10⁶), add DNase I to a 50ml conical tube.
 - a. To thaw purified cells for culture purpose, a total of 100µg is needed.
 - b. To thaw mononuclear cells for cell culture purpose, a total of 300µg is needed.

Note: DNase I should not be used if the cells are used for purification of genomic DNA or if cDNA is for RNA synthesis.

8. Aseptically, using sterile techniques, transfer the cell suspension to the 50ml conical tube, which contains DNase I.

Table 3: Typical Cell Suspension Volumes

Cell Unit	Cell Suspension Volume
$\leq 1 \times 10^6$	1.2 ml
$> 1 \times 10^6$	1.8 ml

9. Rinse the vial with 1ml of 37°C medium. In the 50ml conical tube, slowly add the rinse drop by drop (5 seconds per drop) to the cells while gently shaking the tube.
 10. Next, slowly add the 37°C medium drop by drop (5 seconds per drop) to the cells up to a total volume of 15 to 20 ml (about 10 times or more than the volume of the vial).
 11. Centrifuge the cell suspension at 200g at room temperature for 15 minutes.
 12. Using a pipet, carefully remove most of the supernatant (save the supernatant in a second tube). Leave a few milliliters of the supernatant behind so the cell pellet is not disturbed. Gently resuspend the cell pellet in the remaining few milliliters of medium.
- Note:** DMSO is heavier than medium. Try to aspirate and discard the supernatant soon after centrifugation.
13. While gently shaking the tube, slowly add an additional 15 to 20ml of fresh medium to the tube.
 14. Centrifuge the cell suspension at 200g at room temperature for 15 minutes.
 15. Using a pipet, carefully remove all but 2ml of supernatant. Gently resuspend the cell pellet in the remaining 2ml of medium and count. If the cell count is lower than expected, centrifuge the supernatant saved in Step 11 at a higher speed, count and combine if necessary. The cells are ready for use in your experiment.

Frequently Asked Questions

Q: Why should I add the medium drop by drop?

A: This allows the cells sufficient time for cell re-hydration and gradual loss of DMSO. In addition, adding medium drop by drop prevents osmotic lysis.

Q: Why use room temperature medium?

A: Adding cold temperature medium is damaging to the cells, which cannot support vast temperature exchanges after thaw. Using room temperature medium will prevent further cell viability loss. If your protocol requires using cold temperature medium, we suggest a gradual temperature increase.