

## 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,  $\alpha$ -MEM, or RPMI-1640 containing 10% FBS
- DNase I

### Thawing Cells

- In a 37°C water bath, warm medium (IMDM,  $\alpha$ -MEM, or RPMI-1640) containing 10% FBS.
- 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.
- In a 37°C water bath, quickly thaw the vial for approximately 2 minutes. Be careful not to submerge the entire vial in the water bath. Do not remove the vial until a tiny ice-crystal is left.
- Remove vial from water bath and clean the outside of the vial with 70% alcohol.
- In a biosafety hood, measure the cell suspension volume.
- Count the cells using the Trypan Blue Method. Take a 10 $\mu$ l sample from the vial. Mix 10 $\mu$ l of the sample with 10 $\mu$ l 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 =  $N / 4 \times d \times \text{___ ml} = \text{___} \times 10^4$

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

- (Optional) In order to avoid occasional clumping, when thawing units with large cell numbers (> 100x10<sup>6</sup>), add DNase I to a 50ml conical tube.
  - To thaw purified cells for culture purpose, a total of 100 $\mu$ g is needed.
  - To thaw mononuclear cells for cell culture purpose, a total of 300 $\mu$ g 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.

- 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

- 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.
  - 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).
  - Centrifuge the cell suspension at 200xg at room temperature for 15 minutes.
  - 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.
- While gently shaking the tube, slowly add an additional 15 to 20ml of fresh medium to the tube.
  - Centrifuge the cell suspension at 200xg (1000 RPM on Beckman Coulter™ Centrifuge) at room temperature for 15 minutes.
  - 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.
  - Plate the cells in appropriate-sized dish/flask. Incubate in a humidified incubator specified for tissue culture at 37°C with 5% CO<sub>2</sub>. Your cells are now ready for downstream applications.

For a technical video of this guide visit our **BioTube** page at: [www.youtube.com/user/AllCells](http://www.youtube.com/user/AllCells)