

## Procedure for Thawing Cells

If cells are not immediately used, place vials of cells at  $-152^{\circ}\text{C}$  or in the gaseous phase of liquid nitrogen upon receipt. Cells can be stored at  $-152^{\circ}\text{C}$  or in the gaseous phase of liquid nitrogen for up to 6 months from the date of shipment.

1. Remove the vial of cells from the  $-152^{\circ}\text{C}$  freezer or the gaseous phase of liquid nitrogen and place vial in a  $37^{\circ}\text{C}$  water bath for 1 minute or until the cells begin to thaw. Ensure that the vial is not completely submerged and water does not enter the vial.
2. Remove vial from the water bath and wipe with 70% isopropanol or ethanol to sterilize.
3. Using a sterile 2 mL pipette, transfer cells into a 15 mL tube containing 10 mL of IMDM + 2% FBS or appropriate culture medium.<sup>1,2</sup>
4. Use 1 mL of IMDM + 2% FBS or appropriate culture medium to wash the vial. This ensures that any remaining cells in the vial are recovered. Add to the 15 mL tube.<sup>2</sup>
5. Centrifuge 15 mL tube<sup>2</sup> containing the cells at  $200 \times g$  for 8 minutes.
6. After centrifugation, decant supernatant and discard.
7. Resuspend cell pellet in a known volume of IMDM + 2% FBS or appropriate culture medium ( $\sim 1$  mL) and measure the total volume of cells (total volume is required for cell recovery and viability calculations).
8. Perform a viable cell count using Trypan Blue exclusion.<sup>3</sup>
9. Resuspend cells at the appropriate concentration for your specific assay. If the cells are not used immediately after resuspension, add DNase at  $1\mu\text{g}/\text{mL}$  final to prevent cells from forming clumps.

Note 1: Cells should always be handled within a biological hood using sterile reagents and supplies.

Note 2: When thawing  $> 1$  million cells, transfer cells into a 50 mL tube containing 40 mL of IMDM + 2% FBS or appropriate culture medium.

Note 3: When counting  $\leq 1$  million cells perform a 1 in 5 dilution of the cells by adding  $10\mu\text{L}$  of cells to  $40\mu\text{L}$  of Trypan Blue solution in a small eppendorf tube. When counting  $> 1$  million cells perform a 1 in 20 dilution of the cells by adding  $10\mu\text{L}$  of cells to  $190\mu\text{L}$  of Trypan Blue solution. Mix well. Proceed with cell counts.

**Cells are intended for Research Use Only and are not approved for human or veterinary use or in clinical diagnostic or therapeutic procedures. All human sourced products should be handled at the Biological Safety Level 2 under appropriate safety control procedures to minimize exposure of potentially infectious products.**

---

For Technical Support, please contact ReachBio at 206.420.0300 or email [info@reachbio.com](mailto:info@reachbio.com).