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# Instructions: Thawing Primary Cells

For human and animal blood and bone marrow derived cells

1. Remove the vial of cells from dry ice (upon receipt of shipment), from the -152°C freezer or the gaseous phase of liquid nitrogen and place vial in a 37°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 the outside with 70% isopropanol or ethanol to sterilize.
3. In a Class II Biosafety Cabinet, using a 2 mL pipette, transfer cells into a 15 mL tube containing 10 mL of IMDM + 10% FBS or appropriate culture medium.<sup>1</sup> (all work inside the hood should be performed with sterile instruments and reagents)
4. Use 1 mL of IMDM + 10% FBS or appropriate culture medium to wash the vial. This ensures that any remaining cells in the vial are recovered. Add rinse volume to the 15 mL tube.
5. Centrifuge the 15 mL tube containing cells at 400 x g for 8 minutes.
6. After centrifugation, decant and discard supernatant. If the cells will not be used immediately after resuspension, add DNase at 1µg/mL final to prevent cells from clumping.
7. Resuspend the cell pellet in a small volume of IMDM + 10% FBS or appropriate culture medium (~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 glacial acetic acid and trypan blue.<sup>2</sup>
9. Suspend cells at the appropriate concentration for your specific assay.

**ReachBio cannot guarantee  
cell viability results using  
alternate thawing procedures.**

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<sup>1</sup> When thawing >10 million cells, transfer cells into a 50 mL tube containing 40 mL of IMDM + 10% FBS or appropriate culture medium.

<sup>2</sup> **MANUAL CELL COUNTING** should be performed for cell count accuracy. Cell counting using Automation devices, FACS counting of cells via propidium iodide staining, and other methods are **NOT ACCURATE** and are not recommended.

Please see “**Instructions: Manual Enumeration of Cells**” listed under the [Product Protocols](#) link under the [Support](#) tab of our website. Hard copies are also provided with each shipment of products from our facility.

**ReachBio products are for research use only. Not intended for human or veterinary use, or in clinical or therapeutic procedures.**