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Procedure for Thawing Primary Hemopoietic Cells

1. Warm recommended medium in a 37°C water bath in a conical tube.
2. Wipe the outside of the cryovial with 70% ethanol or isopropanol.
3. In a biosafety hood, twist the cap a quarter turn to relieve the pressure, and then retighten.
4. Quickly thaw the vial of frozen cells in a 37°C water bath for no more than 2 minutes. Wipe the outside of the vial with 70% ethanol.

NOTE: It is important to work quickly in the following steps to ensure high cell viability and recovery. Do not thaw more than 4 amps at the same time.

5. Remove a 20 µL aliquot of cells for counting.

NOTE: Counting cells taken directly from the vial is optional. Counting for step 5 should be performed by another person while someone else is continuing with recovery of the cells.

6. Aseptically transfer the contents of the cryovial to the conical tube with pre-warmed media.

NOTE: Cells from the cryovial should be diluted at least 1:10 ratio in the thawing medium.

7. Centrifuge the cell suspension at 300 X g at room temperature (15 - 25°C) for 10 minutes.

NOTE: Cell loss of up to 30% can be expected during the wash steps.

8. Carefully remove the supernatant from Step 7 with a pipette, leaving a small amount of medium to ensure the pellet is not disturbed.
9. Resuspend the cell pellet by gently flicking the tube.
10. Add an appropriate volume of desired media and perform a cell count.
11. Cells are now ready for use in downstream applications.