

Thawing of PBMC's

If PBMC's are not thawed properly, viability and cell recovery can be compromised and cells may not perform optimally in functional assays. In general, cells should be thawed quickly but diluted slowly to remove DMSO. Cells with DMSO intercalated into their membranes are very fragile, and must be pelleted and handled gently.

1. Warm cRPMI (complete RPMI) to 22°-37°C in a 37°C water bath before beginning thawing procedure.
2. Transfer the tube from -140°C to a 37°C water bath.
3. Hold the tube in the surface of the water bath with an occasional gentle "flick" during thawing. Do not leave the tube unattended during the thawing process. It is important for cell viability that the cells are thawed and processed quickly; thawing takes only a minute or two. When a small bit of ice remains in the tube, transfer the tube to the fumehood. Dry off the outside of the tube and wipe with disinfectant before opening to prevent contamination.
4. Add warm cRPMI drop-wise into the tube containing the cell suspension, slowly over a 30 second period. The final volume should be twice the volume of the cell suspension. Be careful not to exceed the capacity of the tube.
5. Transfer the diluted cell suspension to a 50ml polypropylene tube containing 8ml of warm cRPMI for every vial of cells added.
6. Centrifuge the cells at 1200rpm for seven minutes. Decant the supernatant, and gently flick the tube with a finger to break up the pellet. Resuspend in desired volume of warm cRPMI.
7. Determine cell number and viability.
8. Check for clumps and remove them with a pipettor tip.