Thawing Cryopreserved PBMCs

Peripheral blood mononuclear cells (PBMCs) are a subset of immune cells characterized by the presence of a single round nucleus. These include lymphocytes (T cells, B cells, NK cells), monocytes, and dendritic cells. PBMCs are frequently used in clinical and basic research for the following applications:

- **Drug discovery**
- **Vaccine development**
- **Biomarker identification**
- **Disease modeling**
- **Cancer immunotherapy**

PBMCs isolated from whole blood are often cryopreserved for future use. However, osmotic and temperature changes encountered during freezing and thawing are stressful to cells. Therefore, using the proper techniques and working quickly are essential for higher recovery and viability rates of thawed cells. Here, we provide step-by-step instructions for optimal recovery of cryopreserved PBMCs.

Reagents and Materials

- Cryovial(s) containing frozen cells
- Complete culture medium containing at least 5% fetal bovine serum (FBS) and 1% penicillin-streptomycin
- Sterile serological and transfer pipettes
- Sterile 15ml polypropylene tube
- Water bath at 37 °C
- 70% ethanol
- Tissue-culture treated dishes, plates, or flasks
- Optional: Deoxyribonuclease I (DNase I)

Equipment

- Bench-top centrifuge
- Biosafety laminar flow cabinet (Class II)

Procedure

- ***Preparation of reagents***

1. Prepare complete medium by adding at least 5% FBS and 1% penicillin-streptomycin to your media of choice. Warm culture medium to 37 °C.
2. Sterilize the biosafety cabinet using 70% ethanol.
3. In the biosafety cabinet, aliquot 10 mL of pre-warmed culture medium into a sterile 15 mL polypropylene tube.

- ***Thawing of PBMCs***

1. Transport two cryovials containing PBMCs from the freezer to the water bath on dry ice.
2. Hold the lower half of the vials in a 37 °C water bath for 30–45 s, flicking intermittently. Note:
   - **Do not submerge the vial completely under water.**
   - **Do not thaw more than two vials at a time.**
   - **Do not thaw completely. Allow about 20% of the ice crystals to remain intact.**
3. While 20% of the ice crystal is still visible, take the vials out of the water bath and wipe them with 70% ethanol.
4. Using aseptic technique in the biosafety cabinet, transfer the cell suspension immediately from the cryovial to the 15 mL tube containing culture medium using a sterile transfer pipette. Note: **Dispense the culture media dropwise** from the 15 mL tube to the cryovial to slowly thaw the ice crystals.

The above protocol represents an example of Sanguine's typical processing procedure. Variations of this protocol may occur due to client-specified alterations or contract lab specifications

818-462-8290
learnmore@sanguinebio.com
www.sanguinebio.com

Sanguine

5. Transfer all the contents in the cryovial to the culture medium in the 15 mL tube. Use an additional 1 mL of culture medium to rinse out the cryovial and ensure maximum recovery of PBMCs.

- ***Cell recovery***

1. Gently invert the 15 mL polypropylene tube 3–4 times for even cell distribution and centrifuge at 350 x g for 5 min at room temperature (21°C).
2. Carefully remove the supernatant using a sterile serological pipet. Leave a small amount of media at the bottom to re-suspend the pellet obtained after centrifugation.
3. Gently loosen the cell pellet by flicking. Add 10 mL of culture medium. Repeat steps 1 and 2 in this section.
4. After the second wash, leave a small amount (approximately 0.5 mL) of medium in the tube to resuspend the cell pellet.
5. Optional: If clumping occurs, add DNAse I at a final concentration of 0.1 mg/mL (or 200 Kunitz units/mL) into the cell suspension to minimize or eliminate clumps.

Troubleshooting

Problem	Potential Solutions
Low recovery of PBMCs	▪ After transferring the contents of the cryovial to a tube containing culture medium, rinse the cryovial with an additional 1 mL of culture medium to recover any residual cells. ▪ If there is no visible cell pellet after the first centrifugation, centrifuge the tube again at 350 x g for 10 min. ▪ Ensure that the cell pellet does not get dislodged when the supernatant is discarded.
Low viability of thawed PBMCs	▪ Only use complete culture media that is pre-warmed to 37 °C. Avoid thawing more than two vials at a time because longer processing times can compromise cell viability. ▪ Add culture media to the cryovial drop-wise to avoid stressing newly thawed cells. ▪ Avoid excessive pipetting because this can damage the fragile cells. Instead, gently swirl or invert to mix cells.
Contamination of thawed cells	▪ Use aseptic technique throughout the procedure and work inside a biosafety cabinet. ▪ Do not submerge cryovials under water while thawing cells in the water bath. This may cause water to leak inside the vials and contaminate PBMCs. ▪ Make sure to wipe the outside of the cryovials with 70% ethanol after taking them out of the water bath.

818-462-8290
learnmore@sanguinebio.com
www.sanguinebio.com

Sanguine