User Guide:
How To Thaw Cryopreserved Immune Cells

MILECELL

Product Information

Immune cells, also known as leukocytes, are specialized cells that play a vital role in defending the body against infections, diseases, and foreign substances. These cells circulate in the blood and lymphatic system, forming the core of the immune system. Primary immune cells, including lymphocytes (T cells, B cells, NK cells), monocytes, dendritic cells, and macrophages, are essential components of the immune system and are extensively utilized across diverse research fields, including microbiology, pathology, oncology, vaccine research and development, organ transplantation, and regenerative biology.

At MileCell, we offer purified immune cells, such as Mononuclear Cells (MNCs), etc., sourced from multiple tissues, including Peripheral Blood, Bone Marrow, Spleen, Lymph Node, and Tonsil. These cells are isolated using density gradient centrifugation and shipped in cryopreserved format for optimal viability and convenience.

Product Description	Size Available	Strain
Mouse Peripheral Blood Mononuclear Cells (PBMCs)	5M/10M/25M/50M	C57BL/6N Mouse C57BL/6J Mouse BALB/c Mouse CD-1 (ICR) Mouse
Rat Peripheral Blood Mononuclear Cells (PBMCs)	5M/10M/25M/50M	Wistar Han Rat SD Rat Lewis Rat
Canine Peripheral Blood Mononuclear Cells (PBMCs)	10M/25M/50M/100M	Beagle Dog
Non-Human Primate Peripheral Blood Mononuclear Cells (PBMCs)	10M/25M/50M/100M	Cynomolgus Money Rhesus Monkey
Porcine Peripheral Blood Mononuclear Cells (PBMCs)	10M/25M/50M/100M	Bama Minipig
Rabbit Peripheral Blood Mononuclear Cells (PBMCs)	10M/25M	New Zealand White Rabbit
Feline Peripheral Blood Mononuclear Cells (PBMCs)	10M/25M	Felis Catus
Canine Splenic Lymphocytes	10M/25M/50M/100M	Beagle Dog
Canine Splenocytes	10M/25M/50M/100M	Beagle Dog
Non-Human Primate Splenic Lymphocytes	10M/25M/50M/100M	Cynomolgus Monkey Rhesus Monkey
Non-Human Primate Splenocytes	10M/25M/50M/100M	Cynomolgus Monkey Rhesus Monkey
Mouse Splenic Lymphocytes	10M/25M/50M/100M	C57BL/6N Mouse C57BL/6J Mouse BALB/c Mouse CD-1 (ICR) Mouse
Mouse Splenocytes	10M/25M/50M/100M	C57BL/6N Mouse C57BL/6J Mouse BALB/c Mouse CD-1 (ICR) Mouse
Rat Splenic Lymphocytes	10M/25M/50M/100M	Wistar Han Rat
Rat Splenocytes	10M/25M/50M/100M	SD Rat
Non-Human Primate Bone Marrow Mononuclear Cells (BMMCs)	5M/10M	Cynomolgus Monkey Rhesus Monkey

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Canine Bone Marrow Mononuclear Cells (BMMCs)	5M/10M	Beagle Dog

Storage & Shelf Life

Stable for 5 years at ≤ –150°C.

Thawing Protocol

Equipment, Reagents & Materials Required	
● Biological safety cabinet (BSC)	● 37°C water bath
● Centrifuge	● Portable liquid nitrogen dewar for transporting vials
● Tongs, forceps, timer	● Pipettes & tips
● Pipet-Aid & sterile pipettes	● 75% alcohol and lab wipes
● Sterile conical tubes	● Thawing media: DPBS + 0.5% BSA or HSA + 2 mM EDTA-Na_2

1. Warm thawing media in a 37°C water bath.
2. Remove the cryovial containing the cryopreserved cells from liquid nitrogen storage. Quickly loosen the cap slightly to release pressure within the tube, then retighten. Place it into a 37°C water bath immediately.
3. Hold the cryovial in the water without submerging the cap area. Gently shake the vial while thawing. Avoid contact between the cap and water to minimize the risk of contamination. Remove the vial from water bath when sliver of ice remains (the recommended thawing duration can vary depending on the cell cryovial sizes). Disinfect the vial exterior with 75% ethanol and transfer the cryovial into a BSC.

Recommended Thawing Duration

Cell Number/Vial	10 M/vial	25 M/vial	50 M/vial	100 M/vial
Thawing Time	1 min 45 s	2 min 25 s	2 min 55 s	3 min 15 s

4. Transfer 6~8 mL of thawing media using a 10 mL serological pipette into a labeled sterile 15 ml conical tube. With the same pipette, gently aspirate the cryopreserved cell solution from the cryovial into the conical tube containing the thawing media.
5. Rinse the cryovial and pipette 2-3 times with an appropriate volume of thawing media.
6. Drop-wise, transfer the rinse to the conical tube containing the cells.
7. Slowly add thawing media to a total volume of 15 ml.
8. Aseptically, take sample for cell count and viability analysis.
9. Centrifuge the cell suspension at 400×g for 8 minutes at room temperature (the centrifugation speed and duration can vary depending on the cell and conical tube types).
10. Check the clarity of the supernatant and visibility of a complete cell pellet, and transfer into BSC.
11. Aseptically aspirate the supernatant without disturbing the cell pellet.
12. Flick or tap the tube to loosen the cell pellet.
13. Re-suspend the cells in washing buffer/media gently.

Recommended Resuspension Volume

Cell Number/Vial	10 M/vial	25 M/vial	50 M/vial	100 M/vial
Resuspension Volume	2.0 ml	2.5 ml	5.0 ml	10.0 ml

14. Take sample for cell count and viability analysis.
15. *Optional: Centrifuge the cell suspension at 400×g for 8 minutes at room temperature.

16. *Optional: Check the clarity of the supernatant and visibility of a complete cell pellet.
17. *Optional: Carefully remove the supernatant aseptically without disturbing the cell pellet.
18. Re-suspend the cells in desired media for further applications.

Important Notes

- Cells should be handled following biosafety level 2 (BSL-2) procedures. Always wear personal protective equipment and universal precautions should be utilized when working with these cells. Use aseptic techniques when thawing or handling the cells.
- Thawing cryopreserved cells properly is crucial to ensure the viability and functionality of the cells. Using Good technique and prompt work ensure a high proportion of the cells surviving the procedure.

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