User Guide:
How To Thaw Cryopreserved Human and Animal Immune Cells

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 include lymphocytes (T cells, B cells, NK cells), monocytes, dendritic cells, macrophages, granulocyte, CD34+ progenitor cells, etc. They are essential components of the immune system and are extensively utilized across diverse research fields, including immunology, microbiology, pathology, oncology, vaccine research and development, organ transplantation, and regenerative biology.

At MileCell, we offer a comprehensive portfolio of purified immune cells, including Mononuclear Cells (MNCs), enriched immune subsets such as T cells, B cells, NK cells, Monocytes, Dendritic Cells (DCs), Macrophages (M0/M1/M2), CD34+ Progenitor Cells. These cells are sourced from multiple tissues, including Peripheral Blood, Mobilized Peripheral Blood, Cord Blood, Bone Marrow, Spleen, Lymph Node, and Tonsil, and are available from both human and various animal species. The cells are shipped in cryopreserved format for optimal viability and convenience.

Product Description	Size Available	Species/Strain
Human Immune Cells		
Peripheral Blood-derived		
Human Peripheral Blood Mononuclear Cells (hPBMCs)	10M/25M/50M/100M	Human
Human Peripheral Blood CD3+ T Cells, Positive/Negative Selection	5M/10M/25M/50M/100M	Human
Human Peripheral Blood Naive Pan T Cells	5M/10M/25M	Human
Human Peripheral Blood CD4+ T Cells, Positive/Negative Selection	5M/10M/25M/50M	Human
Human Peripheral Blood Naive CD4+ T Cells	5M/10M/25M	Human
Human Peripheral Blood CD8+ T Cells, Positive/Negative Selection	5M/10M/25M/50M	Human
Human Peripheral Blood Naive CD8+ T Cells	5M/10M/25M	Human
Human Peripheral Blood nTreg Cells	0.5M/1M/2M	Human
Human Peripheral Blood iTreg Cells	2M/5M/10M	Human
Human Peripheral Blood CD14+ Monocytes, Positive/Negative Selection	5M/10M/25M	Human
Human Peripheral Blood CD19+ B Cells, Positive/Negative Selection	5M/10M/25M	Human
Human Peripheral Blood CD56+ NK Cells, Positive/Negative Selection	5M/10M/25M	Human
Human Peripheral Blood Immature/Mature Dendritic Cells (iDCs/mDCs)	1M/2M/5M	Human
Human Peripheral Blood M0/M1/M2 Macrophages	2M/5M/10M	Human
Mobilized Peripheral Blood-derived		
Human Mobilized Peripheral Blood Mononuclear Cells (hmPBMCs)	10M/25M/50M/100M	Human
Human Mobilized Peripheral Blood CD34+ Cells	1M/2M/5M	Human
Bone Marrow-derived		
Human Bone Marrow Mononuclear Cells	5M/10M/25M	Human
Human Bone Marrow CD34+ Cells	0.5M/1M/2M	Human
Cord Blood-derived		
Human Cord Blood Mononuclear Cells (hCBMCs)	10M/25M/50M/100M	Human
Human Cord Blood CD34+ Cells	0.5M/1M	Human
Human Cord Blood CD56+ NK Cells, Positive/Negative Selection	2M/5M/10M	Human
Animal Immune Cells		
Peripheral Blood-derived		
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 Monkey 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

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Bone Marrow-derived		
Mouse Bone Marrow Mononuclear Cells (BMMCs)	5M/10M	C57BL/6N Mouse C57BL/6J Mouse BALB/c Mouse CD-1 (ICR) Mouse
Rat Bone Marrow Mononuclear Cells (BMMCs)	5M/10M	Wistar Han Rat SD Rat Lewis Rat
Non-Human Primate Bone Marrow Mononuclear Cells (BMMCs)	5M/10M	Cynomolgus Monkey Rhesus Monkey
Canine Bone Marrow Mononuclear Cells (BMMCs)	5M/10M	Beagle Dog
Spleen-derived		
Mouse Splenocytes	10M/25M/50M/100M	C57BL/6N Mouse C57BL/6J Mouse BALB/c Mouse CD-1 (ICR) Mouse
Mouse Splenic Mononuclear Cells	10M/25M/50M/100M	C57BL/6N Mouse C57BL/6J Mouse BALB/c Mouse CD-1 (ICR) Mouse
Mouse Splenic Lymphocytes	10M/25M/50M/100M	C57BL/6N Mouse C57BL/6J Mouse BALB/c Mouse CD-1 (ICR) Mouse
Rat Splenocytes	10M/25M/50M/100M	Wistar Han Rat SD Rat
Rat Splenic Mononuclear Cells	10M/25M/50M/100M	Wistar Han Rat SD Rat
Rat Splenic Lymphocytes	10M/25M/50M/100M	Wistar Han Rat SD Rat
Canine Splenocytes	10M/25M/50M/100M	Beagle Dog
Canine Splenic Mononuclear Cells	10M/25M/50M/100M	Beagle Dog
Canine Splenic Lymphocytes	10M/25M/50M/100M	Beagle Dog
Non-Human Primate Splenocytes	10M/25M/50M/100M	Cynomolgus Monkey Rhesus Monkey
Non-Human Primate Splenic Mononuclear Cells	10M/25M/50M/100M	Cynomolgus Monkey Rhesus Monkey
Non-Human Primate Splenic Lymphocytes	10M/25M/50M/100M	Cynomolgus Monkey Rhesus Monkey

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 $EDTA\text{-}Na_2$

1. Warm thawing media in a 37°C water bath.
2. Remove the cryovial containing the cryopreserved cells from liquid nitrogen storage. Place the cryovial on a bed of dry ice to retain consistent temperature when transporting the cryovial to the water bath.
3. Quickly loosen the cap slightly to release pressure within the tube, then retighten. Place it into a 37°C water bath immediately.

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4. 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.
5. 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.
6. Rinse the cryovial and pipette 2-3 times with an appropriate volume of thawing media.
7. Drop-wise, transfer the rinse to the conical tube containing the cells.
8. Slowly add thawing media to a total volume of 15 ml.
9. Aseptically, take sample for cell count and viability analysis.
10. 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).
11. Check the clarity of the supernatant and visibility of a complete cell pellet, and transfer into BSC.
12. Aseptically aspirate the supernatant without disturbing the cell pellet.
13. Flick or tap the tube to loosen the cell pellet.
14. Re-suspend the cells in thawing media gently. Based on the cell count specified in the Certificate of Analysis (COA), calculate the resuspension volume and adjust to obtain a concentration of 1×10^6 cells/mL.
15. Take sample for cell count and viability analysis.
16. *Optional: Centrifuge the cell suspension at 400×g for 8 minutes at room temperature.
17. *Optional: Check the clarity of the supernatant and visibility of a complete cell pellet.
18. *Optional: Carefully remove the supernatant aseptically without disturbing the cell pellet.
19. 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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