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FAQ SERIES

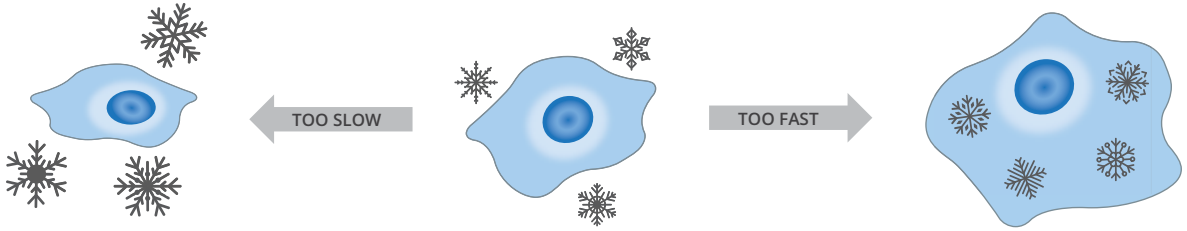
The Science Behind Cryopreservation

Primary cells, such as those isolated from the bone marrow or peripheral blood, are important for a wide scope of research and clinical applications – from studying molecular mechanisms driving disease progression, to developing ex vivo therapies in regenerative medicine. Unlike immortalized cell lines, primary cells have a finite lifespan in culture and can undergo unwanted changes like genetic drift and senescence that impact their functionality *in vitro.* To combat this challenge, cryopreservation is a common practice to preserve and store cells for future use without compromising the *in vivo* characteristics that make them useful for research. While our understanding of the physical and chemical processes that occur during freezing and thawing are incomplete, protocols and reagents for cryopreservation are continuously improving to provide better outcomes. Protecting cells during the transition to lower temperatures—where much of the cellular damage is driven by ice formation—is complex and driven by a number of different factors[1]. Variables in cryopreservation that can affect the success include the selection of cryopreservation medium, cooling rate, freezing equipment, and more. In this installment of our FAQ series, we will take a look at the science behind cryopreservation and key considerations.

WHAT CHALLENGES DO SCIENTISTS FACE WITH CRYOPRESERVATION?

Successful cryopreservation is a balancing act between freezing the cells too fast or too slow – both of which can have deleterious effects on the cells resulting in poor post-thaw cell viability and recovery (Figure 1).

Figure 1. *Effects of cooling rate on cells during cryopreservation.*



Extracellular ice formation increases the solute concentration creating osmotic pressure that draws water out of cells. This changes the intracellular pH and solute concentration, leading to unwanted toxicity effects. Excessive cellular dehydration causes cell shrinkage, physically damaging internal structures.

Intracellular ice formation can occur if the cells are frozen too quickly for water to exit, which can cause mechanical damage (i.e., rupture cellular membranes) when cells are thawed resulting in cell death.

The rate of cooling is a critical aspect to cryopreservation success. It must be sufficiently slow to prevent unwanted intracellular ice formation, but fast enough to mitigate excessive cellular dehydration and physical deformation[1-3]. The inclusion of cryoprotectants (CPAs) in cryopreservation media have also become standard practice in order to enhance cell survival during the freezing process.

WHAT FACTORS NEED TO BE CONSIDERED DURING CRYOPRESERVATION?

While the specifics largely depend on the cell type, a general cryopreservation protocol involves suspending cells in a cryopreservation media containing a CPA, then slowly decreasing the temperature at a uniform and predetermined rate to -80°C. Once the cells are frozen, they may be preserved at ultra-low temperatures (<-135°C), where liquid nitrogen vapor is often used for long-term storage to ensure stability and mitigates loss of viability and function upon thaw. Key considerations during the process are highlighted below:

EQUIPMENT: Either passive cooling devices (i.e., Mr. Frosty) or controlled rate freezer (CRF) units can be used to achieve stepwise temperature decreases. Controlled rate freezer units are actively cooled via liquid nitrogen vapor or electrical mechanisms and are preferred for cryopreservation due to their cooling rate and temperature control.

As the temperature of the cells drops below freezing point, water changes from liquid to crystalline, a process termed ice nucleation[2]. This exothermic reaction releases heat, which can have damaging effects on the cells. In a CRF, the cooling rate offsets this latent heat of infusion, and can be optimized for each cell type.

COOLING RATE: Slow cooling is defined as rates between -0.3°C/min and -2°C/min[2], where a typical cooling rate of about -1 °C/min[2-4] is often employed for a variety of mammalian cell types. The optimal cooling rate at which cells are cryopreserved has a big impact on its long-term viability and is highly dependent on the permeability of the plasma membrane of each cell type.

CRYOPROTECTANTS (CPA): There are different classes of cryoprotectants that can be incorporated into cryopreservation media to protect cells during freezing, however cell-permeating CPAs are most effective for mammalian cells. The most common is dimethyl sulfoxide (DMSO) used at concentrations of up to 10% v/v depending on the application and cell type[1-3]. Other cell-permeating CPAs include glycerol and ethylene glycol (EG). These CPAs enter the cell to replace intracellular water, which helps maintain cellular volume and decrease intracellular ice formation. Outside the cell, CPAs are also secondary solvents for salts, which lowers the solute concentration and osmotic pressure during ice nucleation to mitigate excessive cellular dehydration and damage[2,3]. While these CPAs impart protection during the freezing process, their ability to cross the cell membrane means they have the potential to cause chemical toxicity, which is dependent on both the choice of CPA and the concentration used[4]. Cryoprotectant toxicity remains the main challenge in cryopreservation. Efforts to reduce their effective concentration and to identify alternative CPAs is ongoing[1].

To learn more on how to store and thaw cryopreserved cell products, please read our detailed protocol: allcells.com/resources-and-support/storage-thawing/

It is clear that cryopreservation is a complex process that is influenced by a number of parameters. Protocol and reagent optimization for each cell type is required since not all cells respond equally to a given cryopreservation strategy. The quality and functionality of cells after cryopreservation also depends on the thawing strategy, which goes hand in hand with an optimized cryopreservation protocol[1]. Improper thawing of cryopreserved cells, such as slow execution or incorrect thawing temperature, can undo all the careful preparation during cryopreservation and lead to poor outcomes. Therefore, ensuring proper freezing and thawing of cells ensures high cell viability and recovery post-thaw.

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FOOTNOTES

1. Bojic S, Murray A, Bentley BL, et al. Winter is coming: the future of cryopreservation. *BMC Biol.* 2021;19(1):56. Published 2021 Mar 24. doi:10.1186/s12915-021-00976-8

2. Fuller B, Gonzalez-Molina J, Erro E, Poirier A, Selden C, Awan M, Chalmers S, De Mendonca J. Applications and optimization of cryopreservation technologies to cellular therapeutics. *Cell Gene Therapy Insights 2017;* 3(5), 359-378. 10.18609/cgti.2017.038

3. Jang TH, Park SC, Yang JH, et al. Cryopreservation and its clinical applications. *Integr Med Res.* 2017;6(1):12-18. doi:10.1016/j.imr.2016.12.001

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