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WHY CRYOPRESERVATION IS INTEGRAL TO RESEARCH SUCCESS

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The Importance of Cryopreservation

A big question—perhaps the biggest question—asked of any scientific data is, "Is it reproducible?" Variability throws the validity of scientific data and the conclusions derived from that data into question. One of the leading causes of variability is differences in storage conditions. Improper storage can harm sample integrity, causing degradation or cross-contamination. It can also negatively impact reagents and other experimental components over time, allowing them to lose their efficacy and potency. All of these factors combine to create discrepancies and differences in experimental results, seeding doubt and delaying progress.

What is Cryopreservation?

Cryopreservation uses low temperatures to preserve sample and reagent integrity. Sub-freezing temperatures limit the biological and chemical reactions that take place within cells and tissues, with temperatures of -196°C (that of liquid nitrogen) suspending them altogether[1]. From a tissue standpoint, cryopreservation impedes or inhibits enzymatic actions that would alter, degrade, or destroy 3-D suprastructures and architectures. From a cellular standpoint, it suspends growth, metabolic, and other intracellular mechanisms. Finally, from a molecular standpoint, it limits post-isolation changes to ion, metabolite, and nucleic acid states.

By nature, the freezing process is harmful to cells. Rapid cooling processes produce ice crystals, which cause dehydration and generates mechanical stress. The former results in changes in pH and ionic salt concentrations, leading to denaturation of proteins and membranes. The latter physically alters and deforms cellular morphologies. Conversely, more gradual cooling processes limit ice crystal formation, but can result in intracellular osmotic changes caused by exposure to highly concentrated intra- and extracellular solutions, as well as molecular interactions between cells and extracellular ice[2]. Thawing, by reversing these mechanisms, can likewise shock and damage cells.

Cryoprotectant substances added to samples prior to their initial cryopreservation to limit freeze-thaw damage. Cryoprotectants are divided into diffusible—molecules under 400 kDa that can enter cells—and non-diffusible substances. Commonly used diffusible cryoprotectants include glycerol, 1,2-propanediol, and dimethyl sulfoxide (DMSO), while popular non-diffusible agents include polyvinylpyroldone, hydroxyethyl starch, and certain sugars[1]. Precisely how cryoprotectants work is still somewhat unclear, but they affect the rates of water transport, nucleation, and ice crystal growth[1,2].

Cryopreservation can be Complex

Cryopreservation has become extremely popular in life science laboratories, primarily as a means of storing sample and reagent aliquots for later use. It gives scientists the flexibility to perform multiple assays on the same animal or batch of cells, helping to limit specimen-related variability. At the same time, there is no "one-size-fits-all" answer for cryopreservation. The nature of the sample plays a large part in devising the correct cryopreservation protocol, whether choosing an appropriate cryoprotectant based on tissue composition, deciding between rapid or slow freezing, or selecting the proper storage system (liquid nitrogen vapor phase, liquid nitrogen liquid phase, or ultra-low freezer) based on cellular freeze-thaw damage thresholds[2]. As scientists probe further and obtain more sensitive and precise data, cryopreservation technology and methodology need to keep pace in order to maintain the integrity and trustworthiness of new research findings.

See references on page 7.

Different Cryopreservation Methods

The ability to store samples for later use is key to facilitating increased experimental scope and depth. Having sufficient cryopreservation resources available is necessary for scientists to plan and execute studies that will further our understanding of health and disease. Many different cryopreservation storage formats exist, each with advantages and disadvantages.

Liquid Phase Cryopreservation

Directly immersing samples into liquid nitrogen poured into large cryogenic storage dewar flasks provides the lowest storage temperatures at -196°C—although samples need to remain fully submerged to maintain this temperature. However, direct contact between samples and storage liquid poses a contamination risk, especially if dewars are not cleaned regularly or sample containers are not properly sealed or handled. Despite the low temperatures, researchers have detected bacterial, fungal, and viral contamination within liquid nitrogen tanks—ironically cryopreserved along with the samples[1,2]. Potential contamination sources include the surfaces of sample storage containers, damaged storage containers that expose samples to the liquid nitrogen, or atmospheric particles captured by condensing water particles when dewars are open[3].

Vapor Phase Cryopreservation

Vapor cryopreservation was developed in response to this problem. Here, instead of immersing samples within liquid nitrogen, they are kept above the liquid within the vapor phase created by liquid nitrogen evaporation. The lack of direct contact limits the risk of cross-contamination, but the trade-off is lower storage temperatures of roughly -130°C. Temperatures within the vapor phase vary depending on how far away the surface of the liquid phase is, and as liquid nitrogen evaporates and is refilled, the storage temperatures experienced by samples fluctuate. An additional disadvantage is that because the liquid phase takes up space in the dewar, less space is available for sample storage.

Isothermal Liquid Nitrogen Freezers

Isothermal liquid nitrogen freezers combine the low temperatures of liquid phase storage with the decreased contamination risks of vapor phase storage. Here, samples are placed in a chamber surrounded by a liquid nitrogen-filled jacket. Additional cooling is provided by nitrogen vapor that enters the interior chamber via vents, resulting in a storage temperature around -190°C. The design minimizes contamination by ensuring that samples do not contact liquid nitrogen. It also lessens disruption of the storage environment by allowing jacket refill without opening the storage chamber.

Dry Storage

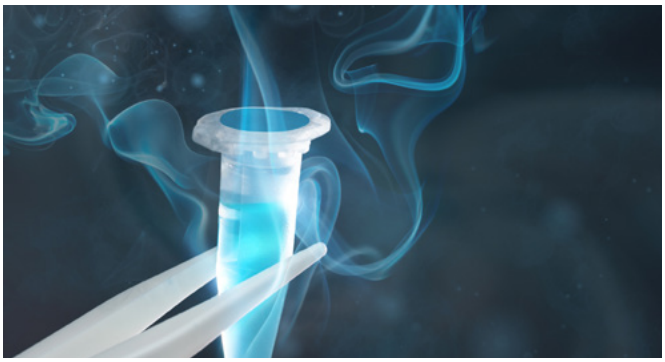
Ultra-low temperature (ULT) freezers offer a potentially more efficient alternative to liquid nitrogen-based storage methods. Many ULT freezers reach temperatures of -80°C, which is not cold enough to fully stop degradation. That said, advances in compressor technology enable the latest freezer designs to reach temperatures as low as -150°C. By providing consistent temperatures and low contamination risks, ULT freezers offer stable storage conditions for short and long-term needs. They also offer larger storage capacities than most dewars. Thanks to these attributes and how easy they are to maintain, ULT freezers are prevalent in life science laboratories around the world. However, freezers come with larger ecological and physical footprints, consume more electricity, generate more heat, and take up more floor space than liquid nitrogen-based storage units. That said, technological progress means that many of the more recent ULT freezer models are smaller and use less energy. Finally, in the absence of good labeling and organizational practices, they can become cluttered. This not only makes it more difficult to locate samples, but also impedes airflow and disrupts storage condition uniformity.

Solutions for Scientist Needs

A variety of cryopreservation storage options, from liquid nitrogen immersion to ULT freezers, exists to serve the needs of life science research. Scientists need to make informed decisions based on their research needs—what samples they are working with today as well as what they will be working with in the future—and their logistical capabilities.

See references on page 7.

Best Practices for Freezing and Thawing Samples



Samples in short- or long-term storage are susceptible to damage caused by freezing and thawing when entering and exiting storage conditions. Freeze-thaw damage introduces the threat of insidious alterations to cellular properties and functions at genetic and molecular levels. This can have significant consequences in a world that increasingly relies on living cells with normophysiological functions, not only as research models, but also as therapeutic instruments. The need for freeze-thaw methodologies that do not stress cells has never been greater[1].

What is Freeze-Thaw Injury?

Sample damage during freezing predominantly occurs at the cellular level, with the cumulative impact across multiple cells manifesting as tissue or structural damage. As temperatures drop, ice crystal formation begins both intra- and extracellularly. Ideally, extracellular ice formation should be more rapid than intracellular ice formation, thus drawing water out of cells through osmosis and limiting intracellular crystallization[1,2]. Excess intracellular ice formation results in cell rupture and the activation of early-stage necrosis mechanisms that become readily visible upon thawing. However, this dehydration process greatly concentrates intracellular solutes—the "solution effect"—potentially leading to protein and nucleic acid denaturation and cellular toxicity[1].

The Right Cooling and Thawing Rate

The cooling rate—how quickly a sample is brought from ambient to storage temperatures—has a tremendous impact on sample viability and integrity. Too fast, and intracellular ice formation is abundant. Too slow, and prolonged exposure to solution effects also activate apoptosis and necrosis mechanisms[1]. Optimal cooling rates can vary dramatically, depending on the nature of the sample and the presence of cryopreservatives, which offset solution effects to some extent[2]. Some trial and error may be necessary, particularly with samples without precedent. Similarly, thawing rate also affects sample integrity to a limited extent. Specifically, rapid thawing improves survival in rapidly cooled samples compared to gradual thawing, but thawing rate minimally influences samples that have been gradually cooled[2].

There are several different strategies when it comes to cooling kinetics. One is to introduce a constant cooling rate over the temperature range where damage can occur (typically the freezing point to around -40°C). Another is to employ "two-step cooling," where cells are rapidly brought to a constant subzero temperature (around -5 to -15°C) and held there to allow water to efflux and limit ice formation. The sample is then rapidly brought to the storage temperature in order to minimize solution effects. Finally, vitrification aims to solidify the sample storage solution without ice formation by increasing its viscosity. In essence, the solution becomes a glass with the physical properties of a solid but the molecular ones of a liquid. Vitrification relies on sufficient cryoprotectant, presenting potential issues with toxicity and osmotic damage. However, if executed properly, it offers freedom from ice formation, solution effects, and cooling/thawing rate optimization (as only a rate sufficient to avoid crystallization would be required)[2].

A multitude of agents with cryoprotective properties have been discovered. While they all protect against freeze-induced injury, the extent of this protection varies considerably. Cryoprotective agents are impacted by cooling rates; for example, glycerol is significantly more effective in preserving cell viability in slowly cooled samples versus rapidly cooled ones. They can also cause cellular damage due to their intrinsic toxicity or their osmotic effects. As such, they must be carefully added prior to cooling and properly removed after thawing[2].

An Ounce of Preparation is Worth a Pound of Cure

Preparing a sample for storage is just as important as how that sample is stored. Much like with storage solutions, scientists need to identify and devise the optimal preparation protocols for their own research needs. Even if the cooling freezer cannot reach a sufficiently cold temperature for long-term storage, samples can be easily transferred to a liquid nitrogen-based system once they are frozen.

See references on page 7.

TROUBLESHOOTING COMMON CRYOPRESERVATION CHALLENGES

Cryopreservation appears simple, but optimizing it for specific research needs can be complicated. Here is a list of key considerations for devising a cryopreservation protocol and storage strategy.

Storage Logistics

Choosing a storage option depends on many factors. What physical and logistical resources are available? Is energy consumption or heat generation an issue? Is cost or space a potential problem? How many samples need to be stored at any given time? Is liquid nitrogen readily accessible?

Consistency

What storage temperatures are required? Is it imperative that storage temperatures not fluctuate? This will depend on the nature of the samples (cells vs. tissues vs. solutions) and how long they will be stored.

Contamination

Some samples are particularly sensitive to microbial presence. Will stored samples be used for analysis only, or will they be introduced into living organisms?

Aliquoting

Proper aliquoting is essential for avoiding unnecessary freeze-thaw cycles. How much of a sample will be required for each assay run? Are individual aliquots accessible without disrupting the storage conditions of the others?

Cryopreservative Agents

Different cryopreservatives have different efficacies. Picking the right one depends on sample sensitivity, intended sample use (will the cryopreservative interfere with data acquisition?), final storage temperature, optimal cool/thaw rate, and potential toxicity.

Cooling and Thawing Rates

Different samples have different sensitivities to rapid cooling and thawing. Optimization requires a combination of literature research and experimental trial-and-error. Technology can assist in executing desired temperature gradient protocols.

Article 1 – The Importance of Cryopreservation

References

1. J. Bakhach, "The cryopreservation of composite tissues: Principles and recent advancement on cryopreservation of different type of tissues," *Organogenesis*, 5(3):119-26, 2009.
2. T.H. Jang et al., "Cryopreservation and its clinical applications," *Integr Med Res*, 6(1):12-18, 2017.

Article 2 – Different Cryopreservation Methods

References

1. V. Mirabet et al., "Use of liquid nitrogen during storage in a cell and tissue bank: contamination risk and effect on the detectability of potential viral contaminants," *Cryobiology*, 64(2):121-3, 2012.
2. G.A. Pessoa et al., "Decontamination of naturally contaminated liquid nitrogen storage tanks," *Rev Bras Zootecn*, 43(5), 244-9, 2014.
3. B.W. Grout, G.J. Morris, "Contaminated liquid nitrogen vapour as a risk factor in pathogen transfer," *Theriogenology*, 71(7):1079-82, 2009.

Article 3 – Best Practices for Freezing and Thawing Samples

References

1. J.G. Baust et al., "Cryopreservation: An emerging paradigm change," *Organogenesis*, 5(3):90-6, 2009.
2. C.J. Hunt, "Cryopreservation: Vitrification and Controlled Rate Cooling," In: Crook J., Ludwig T. (eds) *Stem Cell Banking. Methods in Molecular Biology*, Humana Press, New York, NY, 2017.

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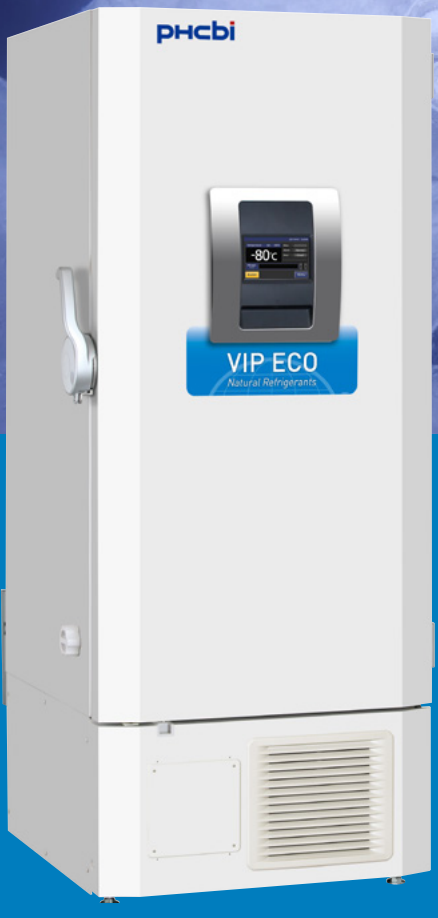

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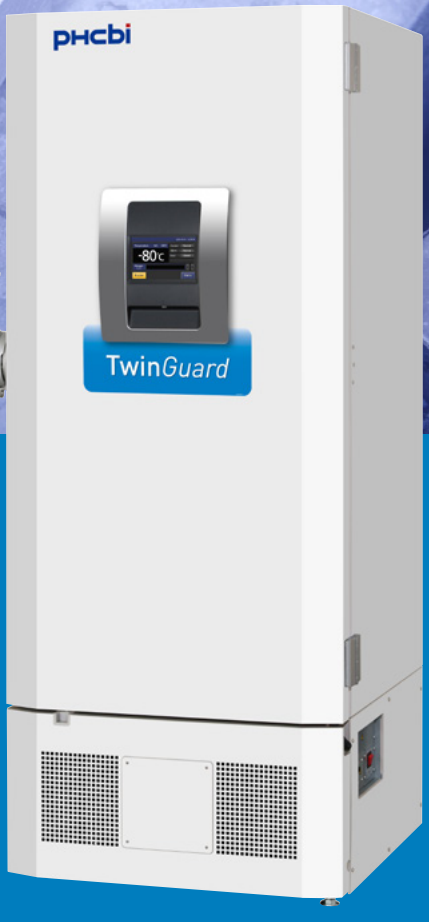

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