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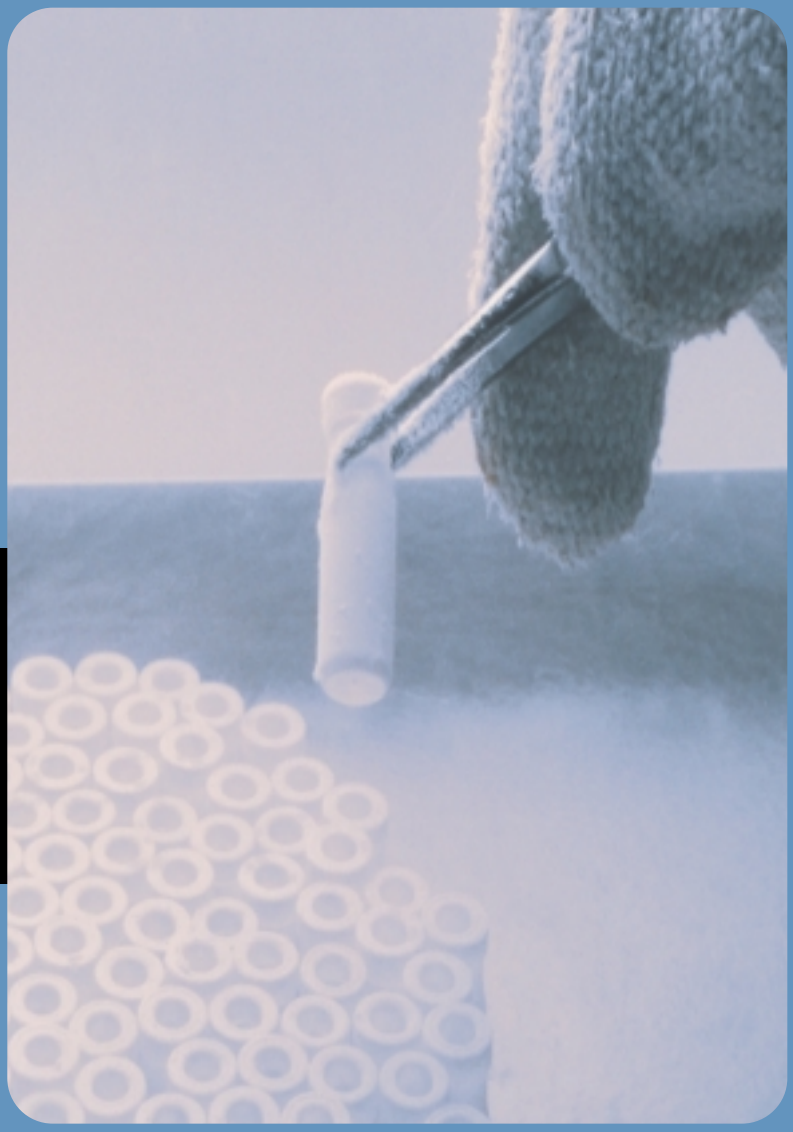
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Cryopreservation Manual



A Guide to Cryopreservation Techniques

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PREFACE

The *Cryopreservation Manual, A Guide to Cryopreservation Techniques* is a practical handbook for scientists who are responsible for an organization's cryopreservation program. This guide presents the following:

- Overview of the cryobiology of cell and tissue preservation
- Comprehensive methodology for selecting and operating cryopreservation equipment

  Controlled rate freezing methods as well as controlled rate freezing equipment selection criteria are addressed. Programming and running a cryopreservation protocol is explained, detailed graphs are included, and data storage is outlined. Various styles of available storage and inventory systems are reviewed. A checklist of questions helps you narrow down the choices that will fit your research requirements.

- Guidance regarding installation and operational qualifications, and safety required in a Good Manufacturing Practices (GMP) laboratory

You will be better prepared to do the following after reading this guide:

- Differentiate various types of controlled rate freezing methods and their purposes
- Develop a protocol for freezing a selected sample in which the potential for cell viability is maximized
- Understand and identify sample phase changes
- Select an appropriate storage system and accessories to meet your required protocol, facility restraints, and budget
- Develop an installation and operational qualification (IQ/OQ) procedure required for a GMP laboratory

Organizations that may employ cryopreservation techniques and find the information in this guide useful include the following:

- Hospital Research Laboratories
- University Research Laboratories
- Government Research Laboratories
- Industrial Research Laboratories
- Pharmaceutical Research Laboratories
- Veterinary Research Laboratories
- Agricultural Research Laboratories
- Tissue Banks
- Commercial Vaccine Operations
- Blood Banks
- Biotechnology Firms

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PART 1: CRYOBIOLOGY AND CRYOPRESERVATION

Cryobiology is the study of the effects of extremely low temperatures on biological systems, such as cells or organisms. **Cryopreservation**—an applied aspect of cryobiology—has resulted in methods that permit low temperature maintenance of a diversity of cells. The objective of cryopreservation is to minimize damage to biological materials, including tissues, mammalian cells, bacteria, fungi, plant cells, and viruses, during low temperature freezing and storage. Cryopreservation provides a continuous source of tissues and genetically stable living cells for a variety of purposes, including research and biomedical processes.

A basic principle of cryobiology is that the extent of freezing damage depends on the amount of free water in the system and the ability of that water to crystallize during freezing. Water is the major component of all living cells and must be present for chemical reactions to occur within a cell. During freezing, most of the water changes to ice, and cellular metabolism ceases. By following published procedures, you can successfully freeze many types of isolated cells and small cell aggregates. However, obtaining reproducible results for more complex tissues, such as heart valves or engineered tissue constructs, or more sensitive cell types, requires an understanding of the major variables involved in tissue cryopreservation.

VARIABLES TO OPTIMIZE

Ice formation initiates in the extracellular environment, resulting in increased salt concentrations as water is removed to form ice. This ice formation results in an osmotic imbalance. Water then leaves the cells by osmosis, and cellular dehydration results. Cryosubstituted cryopreserved tissues demonstrate dehydrated cells sandwiched between extracellular ice domains. Excessive dehydration can be detrimental to cell recovery.

Potentially detrimental effects of dehydration and ice can be minimized by doing the following:

- Controlling the cooling rate by using an appropriate controlled rate freezer
- Using cryoprotective agents in appropriate vehicle solutions
- Maintaining appropriate storage temperatures
- Controlling the rewarming rate

All of these events interact to influence the outcome of cryopreservation. Highest survival is attained by optimizing the series of interrelated variables listed in Table 1.

Table 1.
Key Cryobiological Variables for Optimization

Type of preservation medium

Cryoprotective agents
- Choice
- Concentration

Pre-freeze equilibration schedule

Initiated (i.e., "seeding") or spontaneous freezing

Cooling rate

Termination temperature of controlled rate cooling

Storage temperature

Warming rate

CPA dilution schedule
- Temperature
  - Serial, direct, or osmotic buffer step dilution
  - Presence of serum

Post-thaw culture

*The variables known to have the most significant influence on cell survival are shown in **bold** type. Of these, cooling rate is especially important.*

These interrelated variables are addressed in the following sections: Cryoprotective Agents, Media for Cell and Tissue Cryopreservation, Cooling Rate, Warming Rate, and Post-Thaw Culture.

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CRYOPROTECTIVE AGENTS

Cryoprotective agents (CPAs), or cryoprotectants, and their mechanisms of action have been the subject of many excellent reviews (Karow 1981, Mazur 1984, Brockbank 1995). Glycerol and DMSO are the most commonly employed cryoprotective agents. Fetal bovine serum (FBS) is often used in mammalian cryopreservation solutions, but it is not a cryoprotective agent. Salts, such as magnesium chloride, have been reported to be cryoprotective agents (Karow and Carrier 1969). Dextrans, glycols, starches, sugars, and polyvinylpyrrolidone provide considerable cryoprotection in a variety of biologic systems (Mazur 1981). Cryoprotectants protect slowly frozen cells by one or more of the following mechanisms:

- Suppressing high salt concentrations
- Reducing cell shrinkage at a given temperature
- Reducing the fraction of the solution frozen at a given temperature
- Minimizing intracellular ice formation

Combinations of cryoprotectants may result in additive or synergistic enhancement of cell survival (Brockbank and Smith 1993, Brockbank 1992). Comparison of chemicals with cryoprotectant properties reveals no common structural features. These chemicals are usually divided into the following two classes:

- **Intracellular cryoprotectants** with low molecular weights that permeate cells. Intracellular cryoprotectants, such as glycerol and dimethyl sulfoxide at concentrations from 0.5 to 3 molar, are effective in minimizing cell damage in many slowly frozen biological systems.
- **Extracellular cryoprotectants** with relatively high molecular weights—greater than or equal to sucrose (342 daltons)—that do not penetrate cells. Extracellular cryoprotective agents, such as polyvinylpyrrolidone and hydroxyethyl starch, are more effective at protecting biological systems cooled at rapid rates. These agents are often large macromolecules that affect the solution's properties to a greater extent than would be expected from their osmotic pressure. Some of these non-permeating cryoprotective agents have direct protective effects on the cell membrane. However, the primary mechanism of action appears to be the induction of vitrification (extracellular glass formation).

Ice formation can be eliminated entirely, both within the cells and the extracellular matrix, when cryoprotectants are used in extremely high concentrations (i.e., at least 50% volume/volume) (Fahy 1988).

VITRIFICATION

Preventing freezing requires that the water in a tissue remains liquid during cooling. However, as cooling proceeds, the molecular motions in the liquid permeating the tissue decrease. Eventually, an "arrested liquid" state known as a *glass* is achieved. Vitrification* is this conversion of a liquid into a glass. Glass is a liquid that is too cold to flow. A vitrified liquid is essentially a liquid in molecular stasis. Vitrification is solidification due to increased viscosity rather than to crystallization. The point below which the vitreous material can be considered solid is somewhat arbitrary and depends on the time scale of interest.

Typically, a vitrified material is considered solid when the viscosity reaches ~10^{15} poise. The temperature at which the material can be considered glass is known as the *glass transition temperature*. Glass transition is usually associated with a sudden change in density, which may result in high mechanical stresses and material fractures. These can be avoided if the biomaterial is vitrified but stored at, or just below, the glass transition temperature. Theoretically, vitrification does not have any of the biologically damaging effects associated with freezing.

Vitrification is commonly used to preserve gametes. Vitrification techniques were successfully applied to a variety of complex biological materials—kidney, liver, and heart organ slices, and tissues (i.e., blood vessels, cartilage, skin, cornea). Ice formation was effectively prevented and cell viability was retained.

**Derived from vitri, the Greek word for glass*

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Ice Blockers

A new area of research in cell and tissue preservation is control of ice formation. Nature has produced several families of proteins that permit certain animals and plants to survive in extremely cold climates. These proteins are known collectively as *antifreeze proteins*. It is believed that fish-derived antifreeze proteins act by preferential adsorption to the prism face or to internal planes of ice in such a manner that ice crystal growth perpendicular to the prism face is inhibited. Synthetic ice blockers that modify both ice crystal growth rates and form are being developed at Organ Recovery Systems, Inc. Synthetic ice blockers may be combined with naturally occurring antifreeze proteins and conventional cryoprotectants to develop improved preservation methods in which both the cells and the extracellular matrices are preserved.

MEDIA FOR CELL AND TISSUE CRYOPRESERVATION

Almost without exception, successful cryo-preservation methods begin with a pre-freeze, or pre-vitrification, phase in which the cells are transferred from a physiological environment to a cryoprotective solution. The latter comprises a suitable buffer medium that contains special CPAs essential to minimize freeze-induced injury. Invariably, this pre-freeze phase involves an initial cooling phase, often referred to as the *hypothermic phase*. This hypothermic phase serves two purposes:

- Slows metabolism and minimizes ischemic and hypoxic changes

- Reduces the chemical toxicity of the CPAs

Biological materials may be packaged directly in the pre-cooled cryoprotective solution—0C to 4C (32F to 39.2F)—and then moved to a similarly pre-cooled cryopreservation chamber. Or, a cryopreservation device, such as a controlled rate freezer, may be used to cool the materials from physiological or room temperature to 0C to 4C (32F to 39.2F).

Optimum control of the cells' environment during cryopreservation demands that you consider the chemical composition of the buffer medium used as a vehicle for the CPAs as well as the temperature to which the cells are exposed. Although conventional culture media are commonly used for this purpose, it should not be assumed that a tissue culture medium is an ideal or optimum vehicle solution for exposing cells to low temperatures.

A variety of factors are known to influence cell survival during cryopreservation, but the role of the vehicle solution for the CPAs is often overlooked. It is generally assumed that conventional culture media used to nurture cells at physiological temperatures will also provide a suitable medium for exposure at low temperatures. However, it is now well established in tissue and organ preservation that the ionic and hydraulic balance in cells during hypothermia can be better controlled by using solutions designed to physically restrict temperature-induced imbalances (Taylor and Hunt 1985; Taylor, Elrifai, and Bailes 1996).

Preservation by cooling is achieved by striking a balance between the beneficial and harmful effects of reducing temperature. The most beneficial effect of cooling is the slowing of chemical reactions and, therefore, the decreased demand for oxygen and other substrates and the conservation of chemical energy. Rapid cooling may be harmful due to thermal shock. It has been a common practice in tissue banking to use tissue culture media as the base solution for preservation media. However, there are good reasons why tissue culture media, which are designed to maintain cellular function at normal physiological temperatures, are inappropriate for optimum preservation at reduced temperatures.

Maintaining the ionic and hydraulic balance within tissues during hypothermia can be better controlled in media designed to physically restrict these temperature-induced imbalances. This principle is embodied in the design of organ preservation solutions used for short-term hypothermic storage of kidneys, livers, and hearts without freezing (Taylor 2001) and can be applied equally to the choice of vehicle solution for adding and removing CPAs in a cryopreservation protocol.

Media designed for hypothermic preservation are based on the premise that reducing temperature to near the ice point (0C, or 32F) precludes the need to support metabolism to any significant extent. Under these conditions the correct distribution of water and ions between the intracellular and extracellular compartments can be maintained by physical rather than metabolic means. This is possible because the metabolically-driven

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membrane pumps are inactivated at such hypothermic temperatures, and, in the absence of metabolism, the driving forces for transmembrane ion and water fluxes can be prevented or restricted by manipulating the extracellular environment. Such hypothermic preservation solutions are often referred to as *intracellular-type* solutions because they resemble intracellular fluid in some respects. Principal design elements of the intracellular-type solutions have been to do the following:

- Adjust the ionic balance (notably of the monovalent cations)
- Raise the osmolality by including an impermeant solute to balance the intracellular osmotic pressure responsible for water uptake

A Unified Solution System—UNISOL™

Organ Recovery Systems, Inc. developed the UNISOL concept, which is a unified solution system for both hypothermic storage and cryopreservation based in part on the principle design elements outlined above. The base solution is used as a vehicle solution for a range of additives to derive a system of solutions that are optimized for different applications. A CPA vehicle version of UNISOL has been developed and was recently shown to be superior to EuroCollins solution during the cryopreservation of a variety of cells (Taylor, Campbell, Rutledge, and Brockbank 2001).

➢ *As a practical note, it is recommended that the CPA vehicle solution be prepared as a concentrated solution (typically 3x) to allow incorporation of the cryoprotective agents on a weight percentage basis before diluting to single strength. This preferred method avoids dilution of the salts and buffers that otherwise occurs when CPAs are added to a single strength vehicle solution.*

COOLING RATE

Cooling rate is known to have a most significant influence on cell survival. Controlled rate freezing before long-term storage maximizes viability for a wide variety of cells. Programmed, uniform cooling rates are effective for a variety of freezing applications.

- Aids Research
- Autologous Bone Marrow – Cancer Treatment
- Blood Banking – HLA, Red Cells
- Cancer Research – Tumor Cells
- Genetic Engineering – Cultures
- HLA – (Lymphocytes) Organ Transplant
- IVF – (Embryo, Sperm) Human Infertility
- Microbiology – Viruses, Bacteria, Cell Culture
- Pharmaceutical – Cultures
- Plant Cell Culture – Plant Cells, Seeds
- Repositories – Cell Culture
- Starting Cultures (Food) – Cheese, Beer, Yeasts
- Tissue Banking
  - Skin
  - Bone
  - Heart Valves
  - Corneas
  - Musculoskeletal Tissues

The rate of change from room temperature to 1C to 2C below the solution's freezing point may have a major effect on ultimate viability if the cells are sensitive to thermal shock. In most cases the cooling rate is controlled between 4C and at least -40C (39.2F and at least -40F). During the cooling process, sample freezing may be induced at any point below the equilibrium freezing point. Cooling samples to their freezing point and beyond does not automatically result in freezing the samples at the equilibrium freezing point. Invariably, samples tend to under cool—often referred to as *supercooling*—to a varying degree that depends on the following:

- Cooling rate
- Sample size
- Presence of nucleating agents, which are foreign particles in solution that catalyze the formation of an ice nucleus, initiating the freezing process

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Nucleation represents the onset of a change of state from liquid to crystalline. This process involves the progressive separation of ice from the remaining unfrozen solution, which increases the concentration of solutes as water separates as ice. Initiating freezing is also associated with an energy change. A localized rise in temperature, known as the *latent heat of fusion*, evolves as the system returns to the equilibrium freezing point of the solution (Figure 1).

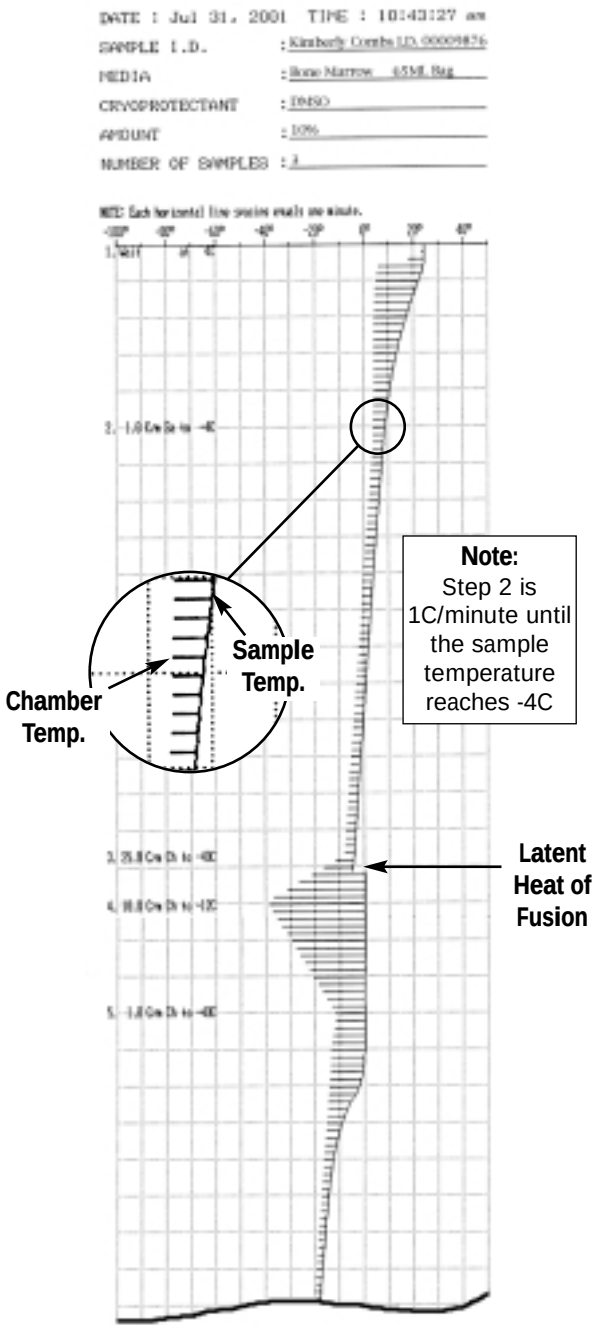


Figure 1. Freezing graph shows heat being released from the sample. Protocol would typically continue to -90C (-130F) or even cooler.

Controlling nucleation and the temperature compensation provided during controlled rate preservation for release of the latent heat of fusion results in improved post-freeze cell viability. This is a major reason to use controlled rate freezing equipment rather than simply to place samples in cold environments.

The controlled rate freezing equipment chamber design is critical to prevent variable degrees of supercooling in multiple samples. Variable supercooling within a single cooling chamber may result in each sample experiencing a different set of conditions. To maintain sample temperature uniformity in Thermo Electron's CryoMed® Freezer, liquid nitrogen injection is distributed evenly into the chamber by an internal fan, minimizing standard deviation to less than 2C during a run. The specially designed air handling system with horizontally directed, recirculating airflow allows greater thermal heat/cooling transfer.

Figure 2 shows the type of cooling profile generated within samples experiencing variable cooling rates as a result of supercooling, freezing, and then the evolution of latent heat. The amount of latent heat evolved is proportional to the degree of supercooling. The sample's post-latent heat-cooling rate is governed by the following:

- The sample's differential temperature at the conclusion of heat evolution
- The temperature of the environment

Samples that supercool to a significant extent undergo a more rapid cooling rate following latent heat evolution (as the sample temperature attempts to catch up with the lower temperature of its environment) than samples that nucleated near their freezing point without significant supercooling. Typically, the environment is a refrigerated chamber controlled at a steady temperature or a cooling machine programmed to cool at a prescribed rate(s).

Since cooling rate is a major determinant of cell viability following cryopreservation, it has proved beneficial to avoid variable degrees of supercooling in multiple samples by deliberately inducing freezing (nucleation) at a point when the samples have cooled a few degrees below their equilibrium freezing point. In this way, a more uniform cooling profile can be achieved for multiple samples.

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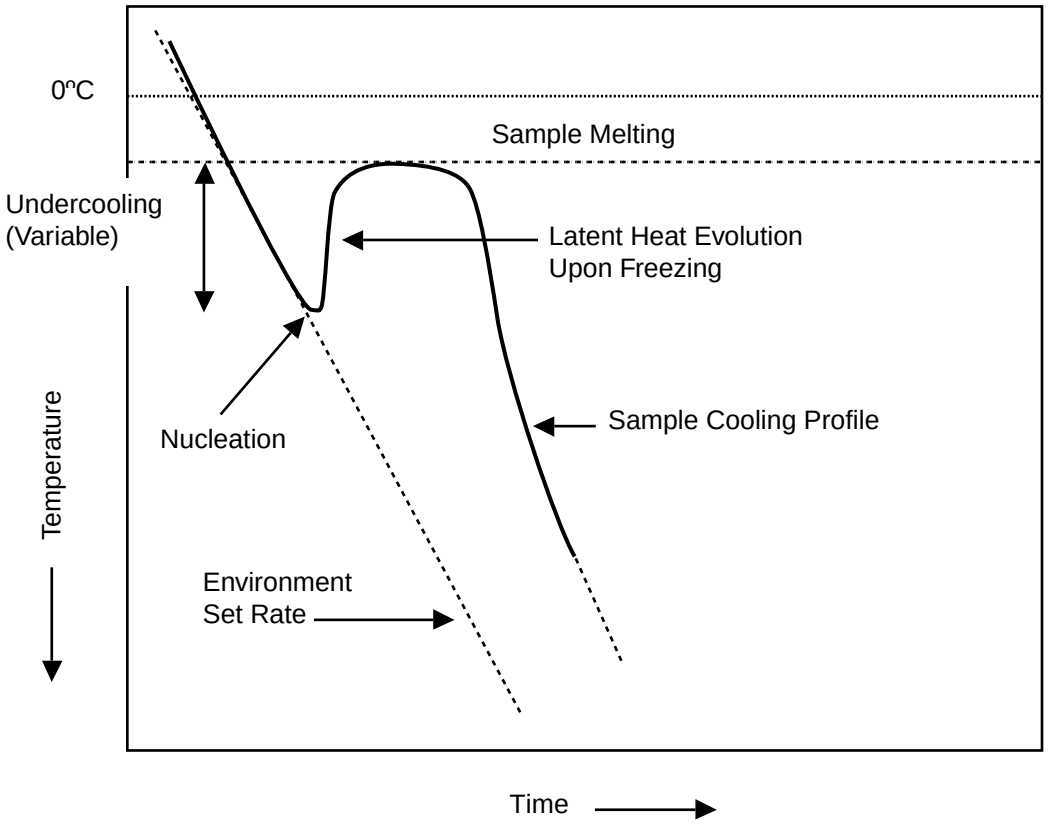


Figure 2. Schematic cooling curves showing the exothermic latent heat evolution of a sample after nucleation

Historically, inducing nucleation by introducing a "seed" ice crystal or other nucleating agent has given rise to the term *seeding*. A variety of other methods have been employed to induce freezing: contact with a cold material, mechanical vibration, and rapid temperature reduction until ice nucleation occurs. The later approach is often used in commercial controlled rate freezers.

Because freezing is an exothermic process, heat release, known as the *latent heat of fusion* or *crystallization*, during ice formation must be conducted away from the material being frozen. The rate of cooling from the sample nucleation temperature to -40C (-40F) or lower is most carefully controlled. We recommend going well below that temperature to -90C (-130F) to reduce the risks of sample warming during transfer from the controlled rate freezer to your storage freezer.

➢ *Note that even though uniform cooling rates are effective for a variety of freezing applications, non-uniform rates may need to be developed for some cells and tissues if adequate viability is not obtained.*

Optimal slow cooling conditions resulting in retained cell viability are defined by the cooling rate that permits some cell shrinkage (dehydration) without the formation of significant amounts of intracellular ice. Tolerances for cell shrinkage and intracellular ice formation vary between cell and tissue types. Ice formation in slowly cooled systems usually initiates in the extracellular solution surrounding the biological material.

Because ice is pure water, as ice formation occurs, the concentration of solute outside the cells increases and the cells begin to lose water by osmosis resulting in cell shrinkage. Cell damage that occurs during freezing may correlate with solute concentration changes. Lovelock demonstrated that hemolysis of frozen erythrocytes could be reproduced by exposure to salt concentrations equivalent to those experienced at successively lower temperatures during the freezing process (Lovelock 1953).

In contrast, some biological systems may be relatively resistant to solute effects. Schneider and Mazur reported that eight-cell embryos were not affected by the high concentrations of salts produced by freezing (Schneider and Mazur 1987).

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They suggested that cellular survival may be determined by the fraction of extracellular solute that remains unfrozen, and that cellular distortion may cause significant damage to cells. In contrast, rapid cooling is generally regarded as harmful to cell viability with certain exceptions, such as red blood cells. Rapid cooling may conveniently be defined as a freezing rate in which little or no cell shrinkage occurs from osmotically driven dehydration. However, at rapid cooling rates the random formation of ultra-structural, intracellular ice occurs. Furthermore, under suboptimal rewarming conditions this intracellular ice may grow and destroy the cells.

Studies on the survival of various mammalian cell types frozen at a variety of rates suggest that the optimal cooling rate usually lies between 0.3C and 10C per minute. Each cell type has a freezing "window" in which the cooling rate provides optimal cell survival. This "window" is narrow at relatively high sub-zero temperatures and becomes increasingly wider as the temperature decreases. Therefore, deviations from established cryopreservation protocols close to zero may be more critical for cell survival than deviations at lower temperatures.

CONTROLLED RATE FREEZING METHODS

A major reason to use controlled rate freezing equipment rather than simply to place samples in cold environments is that the temperature compensation provided during controlled rate preservation for release of the latent heat results in improved post-cryopreservation cell viability. This temperature compensation is provided by a programmed decrease in chamber temperature that both initiates nucleation and subsequently compensates for the release of the latent heat of fusion. The major variables involved are rate of chamber temperature decrease, hold temperature and duration, the rate of temperature increase, and the temperature at which chamber cooling is re-initiated.

The most popular methods of controlled rate freezing on the market today are as follows:

- Liquid Nitrogen (LN_2) Methods
  - Direct temperature feedback
  - Timed pulse
  - Liquid nitrogen submersion, or plunge freezing
- Step Down Method

The use of liquid nitrogen, either by itself or as a source of nitrogen gas, is based on the following unique combination of features:

- Chemically inert
- Relatively low cost
- Non-toxic
- Non-flammable
- Readily available

DIRECT TEMPERATURE FEEDBACK AND TIMED PULSE METHODS

The Direct Temperature Feedback and the Timed Pulse Methods control the chamber temperature, not the sample temperature, though most controlled rate freezers may advance a program step based on the sample temperature. In other words, when the criteria (sample temperatures) are met, the program proceeds to the next step and new criteria.

With the **Direct Temperature Feedback** method, the amount of liquid nitrogen injected into the chamber is determined by Type T thermocouples controlling the chamber and monitoring the sample temperature while comparing actual to program chamber temperatures. The controller automatically adjusts for low pressure liquid nitrogen supply, faster freezing rates, or defective solenoid valves by increasing valve cycles through two independent valves or by turning on a heater to compensate for excessive coolant.

Some Direct Temperature Feedback systems use dual solenoid valves. These valves provide dual injection of liquid nitrogen, permitting faster freezing rates and providing more precise temperature control. Although solenoids wear over time, dual valves provide a back-up for coolant injection. If one valve fails, the failed valve should not produce aberrant results with solenoid wear. The second valve would serve as a back-up.

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With the **Timed Pulse** method, which is often engineered with a microprocessor control system, a solenoid valve(s) meters a timed pulse of liquid nitrogen into the chamber. The amount of liquid nitrogen injected is determined by valve size, tank pressure, valve core wear resistance, and number of solenoid openings. The number of solenoid openings is determined by the temperature-freezing rate programmed.

The Timed Pulse method requires use of a small Dewar flask and a liquid nitrogen Cryo pump system. The pump or heater "boils off" liquid nitrogen, providing consistent pressure and ensuring a measured injection of coolant. This measured amount is used in the calculation that determines the cycle rate of the solenoid valve providing the programmed freezing rate. The controller may not automatically compensate for program deviations.

If a Timed Pulse unit has only one valve and the operator requires faster cooling rates, the manufacturer may provide a larger orifice valve. However, this may result in temperature control problems at slower cooling rates. With a Timed Pulse unit, different cooling curves over time while using the same program may become evident. Although the differences often look like a calibration problem, they may really be the solenoids showing wear.

LN_2 Submersion, or Plunge Freezing, Method

The third method of controlled rate freezing is **Liquid Nitrogen Submersion, or Plunge Freezing**. Samples are loaded into a heat block, and that block is submerged into LN_2. Then, the heater bucks, or tempers, the LN_2 to obtain a controlled freezing rate. This method has been used successfully for small numbers of low volume straws and vials.

Step Down Freezing Method

Step Down Freezing is an often practiced but less automated freezing method. The samples are placed in a refrigerator overnight, transferred to a -70C (-94F) freezer for a period of time, and moved to nitrogen vapor. In some cases, the samples are plunged into liquid nitrogen for permanent storage. This freezing process is time consuming, difficult to repeat and document, and does not provide the controlled cooling rates and ice nucleation associated with a true controlled rate freezer.

WARMING RATE

The rate of rewarming cryopreserved samples can also impact the outcome (i.e., cell viability), but in general this is less critical than the controlled rate of cooling. However, cryobiological variables interact to determine outcome, and the optimum warming rate usually depends on prior cooling conditions.

In most cryopreservation procedures the cooling rate was optimized for rapid rewarming, and in these circumstances slow warming reduces survival. However, successful preservation of mammalian embryos was achieved only after the discovery that slow warming was essential for survival (Whittingham, Leibo, and Mazur 1972). It seems likely that this was due to the cells becoming heavily loaded with solutes, including the cryoprotectant, during slow cooling. This combined with the cells' low water permeability caused osmotic lysis upon thawing. Slow warming allowed sufficient time for cell rehydration and gradual loss of accumulated solutes. Similarly, it is known that red blood cells, which have traditionally been cryopreserved using rapid cooling and warming, can be recovered successfully after slow cooling in the presence of glycerol if they are also thawed slowly (Miller and Mazur 1976).

The prevalence of references to rapid warming rates in cryopreservation publications is mostly due to the common decision to study the cooling rate first, keeping the warming rate constant by sample immersion in a warm water bath. Consequently, subsequent experiments to study warming rate, using the single cooling rate found to be optimal in the initial studies, will be bound to show rapid warming to be optimal! It is only by studying an extensive matrix of cooling and warming rates that the interaction of these two variables can be clearly seen.

Reasons for the interactions are complex. Cells preserved by cooling at a rate optimized for use with subsequent rapid thawing contain small intracellular ice crystals. If such cryopreserved samples are warmed slowly, the small ice crystal nuclei tend to grow by recrystallization, causing cell damage (Farrant, et al. 1977; Farrant, Lee, and Walter 1977b).

The cells can survive rapid thawing because they accumulated relatively little solute during rapid cooling, and recrystallization has little opportunity to occur during rapid warming. Cooling rate and warming rate cannot be optimized independently.

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POST-THAW CULTURE

After thawing, the final stage of any cryopreservation procedure is to return the cells to their normal environment—isotonic physiological solutions or body fluids that lack cryoprotective agent. This imposes similar osmotic problems to those induced by thawing because an osmotic gradient is again established across the plasma membrane.

Balancing solute loss and water influx is essential if cell swelling is to be kept within tolerable limits. In practice, this involves either of the following methods:

- Diluting the cryoprotectant incrementally, at appropriate time intervals, to maintain cell volume below the threshold for damage

- Diluting the cryoprotectant in the presence of a non-permeating solute (e.g., sucrose) that prevents cell swelling as solutes diffuse out of the cells

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PART 2: METHODOLOGY FOR SELECTING AND OPERATING CRYOPRESERVATION EQUIPMENT

This section provides guidance about selecting a controlled rate freezer and accessories, developing freezing protocols, and choosing a storage and inventory control system (e.g., racks, canisters).

SELECTING A CONTROLLED RATE FREEZER

Many purchasing considerations need to be evaluated when selecting a controlled rate freezing system (Figure 3). A checklist of key considerations follows.



Figure 3. CryoMed Freezers are designed specifically for controlled rate freezing

- **System Size.** Determining chamber size is based on your current needs in terms of sample size and quantity required to freeze, as well as future requirements. Theoretically, a larger chamber would consume larger amounts of liquid nitrogen than a smaller chamber. In reality, liquid nitrogen consumption is difficult to calculate. Larger chambers, such as the 1.7 cu. ft. (48.1 liters) units offered by Thermo Electron, often offer many advantages.

  For example, you can freeze large samples or larger quantities at one time, which may result in a cost savings; and you may freeze a greater variety of samples when sharing the unit. Usually organizations cryopreserving samples such as stem cells, skin grafts, heart valves, and other large volume samples, choose a larger capacity chamber. Smaller sizes, such as 0.6 and 1.2 cu. ft. (17.0 and 34.0 liters) units, provide the advantage of requiring less lab bench space.

- **Quality—Manufacturer and Product.** Many manufacturers define, design, and build their products to conform with quality guidelines (e.g., Continual Improvement). Consider the manufacturer's quality standards, such as whether the company is ISO 9000 Certified. The ISO 9000 series is a set of international requirements for establishing and maintaining quality management systems. Continued compliance is assured by ongoing internal audits and periodic audits by the assessing agency.

  Recommendations, specifications, and certification can guide you regarding product performance. Find out if the unit construction and electrical characteristics comply with Underwriters Laboratories, CSA International, and International Electrotechnical Commission requirements.

  UL Listing, CSA Certification, and the CE Mark attest to the electrical and mechanical safety of equipment. Application of the CE Mark also means that the equipment has been tested for compliance with international requirements related to emission of, and susceptibility to, RF energy and electrostatic discharge. Controlled rate freezers undergo stringent testing before they carry the UL, CSA, and CE markings. Manufacturers should be able to provide copies of UL, CSA, and CE documentation for compliance verification.

- **Support—Sales and Service.** Many times it is the customer support you receive before and after a sale that differentiates companies. For example, during the information gathering phase you may receive explanations of the product's features and benefits, a chart of detailed specifications, and answers to questions that will help you decide what equipment and accessories you need. After the equipment is on hand, after-sale care may include a request that you complete a follow-up survey so the company can gain honest feedback and improve processes, if necessary.

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Many manufacturers have distributors for their products, others have a direct, factory-trained sales force. Multiple modes of distribution are typical. Find out if the sales personnel are readily available to answer your questions and whether they can help set up your equipment when it arrives. Also verify that the manufacturer is able to ship the controlled rate freezer(s) to meet your timeframe.

Everyone hopes that a product won't require service. However, periodic maintenance or fine tuning will keep your equipment in peak operating condition. Therefore, ask the sales representative or distributor about after-sale service and determine the following:

- Was the unit designed for easy access to components?
- Whom do you call for assistance?
- What are the hours of operation?
- Are technicians available by phone to help you troubleshoot problems, if necessary?
- What are the warranty conditions?
- Are extended warranties and maintenance agreements available?

• **Price.** Everyone wants the best deal possible when making a purchase. Is the price competitive? If it is not, try to identify the reason(s). Are there additional features on one unit that are optional on another? Is the product more dependable or expected to have a longer life? Ensure that you are comparing "apples to apples."

Controlled Rate Freezer Accessories

Many accessories, such as racks, bag freezing presses, cryo gloves, vials, thermocouple sensors, tongs, cap codes, markers, etc., are available for use with controlled rate freezers (Figure 4).

Examples of rack types include cane freezing racks, vial racks, rack holders, straw racks, cord blood racks, skin racks, and bag freezing presses.

To optimize the use of chamber space, some racks are designed for specific sizes of controlled rate freezers, while other racks may fit a variety of sizes. For example, one Thermo Electron 1.2/2.0ml Cryo Rack Holder is designed for both the 1.2 and 1.7 cu. ft. (34.0 and 48.1 liters) CryoMed Freezers, and a 77 position cane freezing rack is designed for all three sizes of CryoMed Freezers. Rack requirements are based on the type of sample you are freezing and the size of the controlled rate freezer.

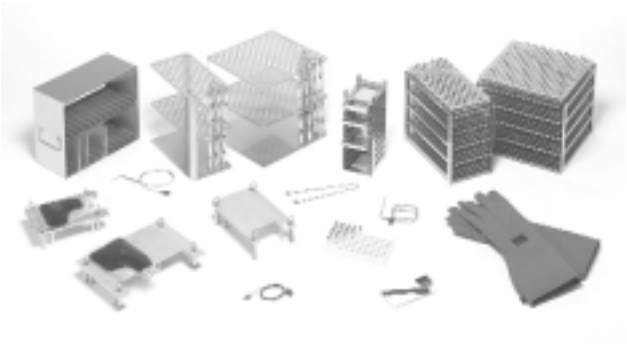


Figure 4. Examples of freezing chamber racks and rack holders, bag presses, and other accessories

DEVELOPING FREEZING PROTOCOLS

After selecting a controlled rate freezing system, you need to develop the protocols for your cells or tissues, and program the protocols into your new equipment. If the protocols are unknown, some experimentation must take place to determine the steps required to obtain the desired level of cell viability or tissue function. Some manufacturers offer preset protocols, or programs, that may produce acceptable viability or at least provide a starting point for your protocol development. Figures 5 through 10 provide examples of six programs that are factory preset on Thermo Electron's CryoMed Freezers.

After you develop a protocol by selecting a preset program and evaluating your individual results, or by defining your own protocol that maximizes cell viability within your sample, you are ready to control rate freeze your first sample batch.

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PROGRAM #1

Commonly used for 2.0ml sample size, resulting in a 1C rate from nucleation to -40C (-40F) and a 10C per minute cooling rate to a -90C (-130F) end temperature

Step 1	Wait at 4C (39.2F)
Step 2	1C per minute to -4C (24.8C) Sample
Step 3	25C per minute to -40C (-40F)
Step 4	10C per minute to -12C (10.4F)
Step 5	1C per minute to -40C (-40F)
Step 6	10C per minute to -90C (-130F)
Step 7	End

Figure 5. Preset Program #1 for freezing 2.0ml samples

PROGRAM #2

Commonly used for very small (96 well) sample size, resulting in a 1C rate from nucleation to -40C (-40F) and a 10C per minute cooling rate to a -90C (-130F) end temperature

Step 1	Wait at 4C (39.2F)
Step 2	1C per minute to -40C (-40F) Sample
Step 3	10C per minute to -90C (-130F)
Step 4	End

Figure 6. Preset Program #2 for freezing very small sample sizes

PROGRAM #3

Commonly used for straw size samples, resulting in a slow rate to -7C (19.4F), a 5 minute hold for manual cell nucleation, a 0.3C rate to -35C (-31F), and then a 0.1C per minute cooling rate to a -37C (-34.6F) end temperature

Step 1	Wait at 20C (68F)
Step 2	1C per minute to -4C (24.8F)
Step 3	0.5C per minute to -7C (19.4F)
Step 4	5 minute hold time at -7C (19.4F)
Step 5	0.3C per minute to -35C (-31F)
Step 6	0.1C per minute to -37C (-34.6F)
Step 7	End

Figure 7. Preset Program #3 for straw size samples

PROGRAM #4

Commonly used for hemopoietic stem cells that are 65-100ml in size, resulting in a 1C rate from nucleation to -45C (-49F) and a 10C per minute cooling rate to a -90C (-130F) end temperature

Step 1	Wait at 20C (68F)
Step 2	1C per minute to -6C (21.2F) Sample
Step 3	25C per minute to -50C (-58F)
Step 4	10C per minute to -14C (6.8F)
Step 5	1C per minute to -45C (-49F)
Step 6	10C per minute to -90C (-130F)
Step 7	End

Figure 8. Preset Program #4 for freezing hemopoietic stem cells

PROGRAM #5

Commonly used for skin packets, resulting in a 1C rate from nucleation to -35C (-31F) and a 10C per minute cooling rate to a -90C (-130F) end temperature

Step 1	Wait at 4C (39.2F)
Step 2	1C per minute to -4C (24.8F) Sample
Step 3	20C per minute to -40C (-40F)
Step 4	10C per minute to -10C (14F)
Step 5	1C per minute to -35C (-31F)
Step 6	10C per minute to -90C (-130F)
Step 7	End

Figure 9. Preset Program #5 for freezing skin packets

PROGRAM #6

Commonly used for hemopoietic stem cells that are 65-100ml in size, resulting in a 0.5C rate from nucleation to -20C (-4F) and a 1C per minute cooling rate to a -80C (-112F) end temperature

Step 1	Wait at 4C (39.2F)
Step 2	1C per minute to -4C (24.8F) Sample
Step 3	20C per minute to -45C (-49F)
Step 4	10C per minute to -10C (14F)
Step 5	0.5C per minute to -20C (-4F)
Step 6	1C per minute to -80C (-112F) Sample
Step 7	End

Figure 10. Preset Program #6 for freezing hemopoietic stem cells (variation of program #4)

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BEGINNING THE FREEZING OPERATION

Preparing the Freezing Chamber. Liquid nitrogen only transfers at liquid nitrogen temperatures, -196C (-320F); therefore, it is a good practice to begin cycling the chamber to cool it before initiating the freezing run. Pre-cool the freezer chamber to 4C (39.2F), which is ice bath temperature, while preparing your samples for batch freeze. A different initial chamber temperature can be used if validated for your samples. The objective is to equilibrate the chamber and sample temperatures before beginning the controlled rate freeze.

Preparing the Samples. After preparing samples in an ice bath at 4C (39.2F), transfer them to the pre-cooled chamber. A different sample accumulation temperature can be used if validated for your samples.

Freezing the Samples. After the samples are prepared and placed in the freezing chamber, the samples and chamber must be allowed to cool to the 4C Start Temperature. When the sample temperature reaches the start temperature or at least within 2C of the Start Temperature, advance the program to activate the automated steps for protocol completion. Preset programs stored in CryoMed Freezers begin with a Wait step. This step ensures that you maintain control of when the remaining steps of the controlled rate freezing process begin (i.e., Run is pressed a second time to initiate the remaining steps). The major phases of a cryopreservation program are as follows:

1. **START TEMPERATURE.** The start temperature is usually the same as the sample temperature, before the samples are loaded into the freezing chamber. Usually the samples are at room temperature—22C (71.6F)—or at ice bath temperature—4C (39.2F). When temperatures below room temperature are used, both the samples and the chamber may warm up during sample transfer to the controlled rate freezer chamber. The chamber and samples should reach the start temperature before the automated portion of the program is initiated.

2. **LIQUID PHASE COOLING.** This is the phase during which the liquid sample is cooled before nucleation. A typical cooling rate is between 0.2C per minute and 10C per minute.

3. **SUPERCOOLING.** This phase refers to the temperature drop, just before the liquid-to-solid phase change, below the freezing point of the freezing medium. Typically, it is 3C to 4C (37.5F to 39.2F) below the freezing point but may be much lower if nucleation is allowed to occur spontaneously.

4. **PHASE CHANGE.** The beginning of the liquid-to-solid phase change process is characterized by a rapid increase in temperature from the supercooled temperature to the freezing temperature. The chamber temperature is dropped rapidly to minimize the sample temperature rise during the phase change. The magnitude and duration of the drop depend on the sample volume and geometry. This rapid cooling provides a heat sink for the latent heat of fusion.

5. **SOLID PHASE I FREEZING.** The cooling rate selected for Solid Phase I Freezing is independent of the liquid phase cooling rate. This rate is determined by the operator, as required, and is often the same as the liquid phase cooling rate of 1C per minute.

END SOLID PHASE I FREEZING. This is the final temperature achieved in the Solid Phase I Freezing cycle. It is usually set between -30C and -50C (-22F and -58F), as required by the operator.

The protocol freezing rate may be increased to shorten the duration of the freezing cycle after Solid Phase I Freezing is completed. A commonly used rate is 10C per minute to an End Solid Phase II Freezing temperature of between -80C and -90C (-112F and -130F). This provides adequate temperature security by preventing sample warming above the End Solid Phase I Freezing temperature during sample transfer from the controlled rate freezer to permanent storage.

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STORING CRYOPRESERVED SAMPLES

A commonly asked question is "Why use expensive mechanical storage freezers or liquid nitrogen storage systems for long-term sample storage?" The answer is that the temperature at which frozen cells are stored may have major effects on product shelf life. Typically, the lower the temperature, the longer the viable storage period. For example, while many samples may be stored at -70C (-94F) for months or even years, the chemical reactions responsible for cellular deterioration are not completely halted at this temperature. Samples at temperatures below the "glass transition of water" or -130C (-202F), where it is said that biological time has stopped, may be stored for millennia (Karow 1981, Mazur 1984).

Degradative processes may occur at and above the solution's glass transition temperature. For example, heart valve leaflets retain protein synthesis capabilities for at least two years when stored below -135C (-211F); however, protein synthesis is reduced after storage at temperatures warmer than -100C (-148F). Immersing tissues directly into liquid nitrogen for as little as five minutes may result in tissue fractures (Adam, et al. 1990).

The formation and the disappearance of fractures in experimental cryopreservation solutions depend on the interaction of several factors, particularly the following (Wolfingbarger, et al. 1991; Kroener and Luyet 1966a & 1966b; Rubinsky, Lee, and Onik 1987; Rajotte, et al. 1977):

- Mechanical properties of the material
- Solute concentration
- Temperature gradients
- Overall temperature
- Rate of temperature change

In addition to a very long viable storage period, another advantage of a liquid nitrogen storage system is that electrical power is not required to maintain your samples in a safe environment. Power is only needed to support the system's electronic features (i.e., auto-filling, monitoring, alarms). This manual addresses liquid nitrogen storage only.

LN_2 Storage Phases

The two liquid nitrogen storage phases are Liquid and Vapor. Figure 11 illustrates how samples can be stored in the vapor phase or in the liquid phase.

Liquid phase storage offers a uniform temperature of -196C (-320F). Problems associated with this method of storage are that packaging materials used (e.g., cryovials) may leak and allow liquid nitrogen to enter the package. This introduces the risk of both sample contamination and cross contamination with microbes. Furthermore, when these packages are retrieved from storage, the trapped liquid nitrogen expands to gas as it is warmed and may cause a small explosion unless the gas is allowed to escape. Glass vials or packages with small breaks may also explode.

➢ *Experienced researchers have learned that opening the vial lid immediately to diffuse the pressure alleviates this threat. There is minimal risk to the operator providing standard safety precautions are followed. Part 3: Safety and Quality addresses liquid nitrogen safety.*

Vapor Phase Storage offers a temperature gradient, not a uniform temperature. For instance, in a wide-mouth Dewar it is not unusual to have between a -75C to -100C gradient from the liquid to the vapor just under the lid. Raising the liquid level or adding a conductive material liner, such as aluminum, will reduce the gradient. However, the gradient cannot be eliminated unless the Liquid Phase is instituted.

➢ *To minimize temperature cycling and gradients within the upper sections of the storage container, it is recommended that your container be filled with the maximum number of racks or containers. This, combined with a temperature or liquid level monitoring device, should minimize temperature fluctuations within the storage chamber and over time.*

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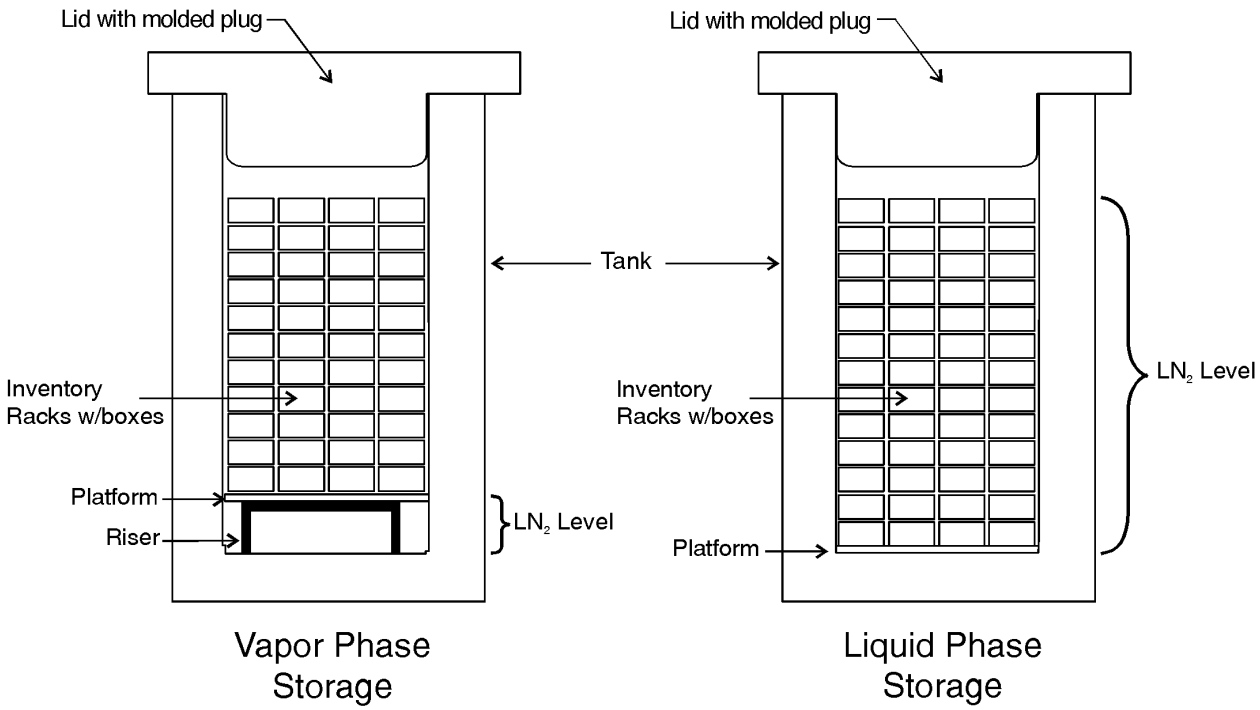


Figure 11. Examples of vapor phase and liquid phase storage in an LN_2 storage system

Defining Your Storage Requirements

It is important to understand your needs before deciding what type of equipment to buy and which product features to require for optimal performance of your cryopreservation equipment. General questions to answer are as follows:

- What products are to be stored?
- Eventually, how many samples will you have?
- Do you require a canister type or a rack type of inventory system?
- Will you store your samples in liquid or vapor phase?
- What will your liquid source be? Bulk? Liquid cylinder? Dewar?
- What temperatures are required for your protocol?
- Will you require an auto-fill system (i.e., liquid nitrogen is automatically injected when it is below a specific level in the storage system)?
- Will you monitor the system's operation and require temperature documentation?

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Selecting an LN_2 Storage System or Container

The range of liquid nitrogen storage options includes the following:

- Complete **long-term storage systems** (Figure 12) with microprocessor liquid level controls and alarm systems and multiple styles of inventory control systems



Figure 12. LN_2 storage systems for continuous long-term storage

- **Portable storage containers** that include racks or canisters (Figure 13)



Figure 13. Portable LN_2 storage containers with canisters, racks, and optional roller bases

- **Shipping containers** that are convenient for transporting samples (Figure 14)

Your LN_2 storage selection should be based on the four Cs—**C**ost, **C**onsumption (liquid nitrogen loss rate), **C**apacity, and **C**ontrol. Typically, the smaller the neck diameter, the less the consumption, the less capacity, and the unlikelihood of electronic auto-filling or liquid level control.



Figure 14. Dry shippers for easy transport

Cost. A primary consideration is whether the equipment is priced competitively. If it is not, determine the reason(s), such as additional features, better quality (e.g., construction and materials), or uniqueness. A common method of determining value is to evaluate cost per vial, which is calculated as follows: unit price ÷ unit capacity.

Consumption. Static evaporation rate is used to evaluate a unit's vacuum integrity after the manufacturing process. The vacuum is pumped down on a unit, and then liquid nitrogen is added. The boil off is allowed to stabilize overnight, and the unit is weighed. The unit is weighed again after 24 hours, allowing the evaporation rate to be calculated and the pass/fail status to be determined. Many laboratory professionals attempt to use this figure as their daily liquid nitrogen consumption level, and they prepare their budgets based on this calculation. Those who do are usually over budget early in the year because they have not considered the following additional items that affect consumption:

- New supply tanks loose approximately 2% of their volume daily (per manufacturing specifications). However, most tanks have been in use for awhile and may consume slightly more than 2% of their volume daily.

- Inventory systems and samples, which are not present during static evaporation testing, consume liquid nitrogen as heat is removed from them.

- The filling process consumes liquid. Because nitrogen only transfers at nitrogen temperatures, everything in the filling path must be cooled before liquid transfer will result. Until then you are only adding warm gas.

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- Daily system use, including lid openings and inventory retrieval and placement, causes liquid nitrogen loss.

- Liquid phase versus vapor phase storage affects consumption. The higher the liquid levels, the colder the temperature at the top of unit. The colder the temperature maintained, the greater the amount of liquid nitrogen consumed.

➢ *A "rule of thumb" for liquid nitrogen consumption in a large capacity—16.0" to 40.0" (40.6cm to 101.6cm) diameter—storage unit is approximately 180 liters every 7 to 10 days.*

Capacity. Liquid nitrogen storage freezers must be used with a complete racking system (inventory control system) in place to prevent undesirable temperature fluctuations and gradients. Therefore, it is best to select a small, medium, or large unit based on anticipated need. Examples of sizes include the following:

- 16.0", 24.0", 31.0", and 39.5" (40.6cm, 61.0cm, 78.7cm, and 100.3cm) diameter tanks for long-term storage

- 14.5" to 22.0" (36.8cm to 55.9cm) diameter portable containers for short-term storage

- 7.3" to 15.0" (18.5cm to 38.1cm) diameter dry shippers

Control (Temperature, Auto-Fill, and Monitoring). Typically, storage containers with neck diameters of less than 16.0" (40.6cm) are manually filled. Some models include a low-level alarm system. As the neck diameter increases, so does the capacity and likelihood of electronic controls for alarms and liquid levels. Pressure differentiation, rather than thermocouple sensing, has become the standard for measuring liquid level. It is a very reliable method of monitoring tank conditions and performance. Most manufacturers offer audible, visible, and remote alarms to help researchers monitor their liquid nitrogen storage units and recording devices for data validation and documentation.

You must weigh your own perceived value of electronic features, such as battery back-up and a defogger. Battery back-up allows continued liquid level and temperature monitoring during power outages for up to 72 hours. It is unclear how many times the battery would be able to energize the solenoid for refilling liquid nitrogen during that 72 hour period. A defogger switch makes it possible to clear the LN_2 vapor in the storage container for visibility and easy inventory retrieval. Some manufacturers include separate switches for manual fill and a defogger, while others offer both of these functions in one single manual fill switch.

Overall, the benefits need to be weighed against the cost of each feature because power is only required to support the electronic features (i.e., auto-filling, monitoring, and alarms) and is not required to safely store your samples. Power outages only demand that you revert to a manual filling mode of operation should power remain off for any length of time.

Selecting an Inventory Control System

Inventory control systems allow you to take full advantage of an LN_2 storage system. Racks, risers, canisters, and frames are designed and made available for optimum storage of your biological specimens (Figure 15).



Figure 15. Examples of inventory control systems and other cryopreservation accessories

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Most manufacturers offer inventory systems in vapor or liquid phase capacities for 2.0ml, 4.0ml, and 5.0ml samples. Typically, vertical racks, which allow you to work conveniently in the vapor or liquid phase, are available in 11 or 13 box configurations, each with 100-cell dividers. Racks are commonly shipped complete with boxes, dividers, and locking rods. Risers are available for vapor phase storage. Custom inventory systems are often available.

Storage capacity is based on the cryo tank size and inventory configuration/layout. For example, a 16.0" (40.6cm) diameter CryoPlus Storage System can hold 6,318 vials (2.0ml) in liquid phase storage when the following is selected: 6 arrowhead racks, each with 13 cardboard boxes (2.0" high) that have 81 cells per divider.

13 boxes x 81 cells/divider = 1,053 cells
1,053 cells x 6 racks = 6,318 cells (2.0 ml vials)

A 39.5" (100.3cm) diameter tank can store 36,400 vials (2.0ml) in liquid phase storage when the following is selected: 28 vertical racks, each with 13 cardboard boxes (2.0" high) that have 100 cells per divider.

13 boxes x 100 cells/divider = 1,300 cells
1,300 cells x 28 racks = 36,400 cells (2.0ml vials)

Advantages of inventory rack systems include easy organization of your samples; the ability to grasp the rack handle and pull out one rack at a time, leaving one hand free to retrieve your sample(s); and minimizing exposure to ambient temperature conditions.

CONCLUSION

An important general conclusion that has emerged from cryopreservation studies is that different types of cells have different requirements for optimum preservation. It is clear from the considerations outlined in this manual that successful, long-term cryopreservation of cells depends on developing procedures in which optimum conditions for a considerable number of interrelated variables are defined. Procedures have already been defined for a wide variety of cells. However, intriguing and sometimes formidable problems still exist when attempting to cryopreserve complex multicellular tissues. For example, extracellular ice formation, which is generally innocuous in single cell suspensions, presents a significant hazard in multicellular tissues but can be avoided by vitrification. Cryopreservation of multicellular tissues is an area of ongoing research.

At this time, you should be able to preserve most cell types and some selected tissue types by combining the following:

- Your knowledge of, and/or experience with, the chemical control of ice formation
- The selection process for state-of-the-art equipment that effectively controls cooling and warming conditions
- Selection and inventory configuration of reliable equipment to store your preserved samples until they are needed
- Knowledgeable sales associates to assist you with your equipment selections
- Availability of process procedures to facilitate the fulfillment of regulatory requirements and improving product quality

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PART 3: SAFETY AND QUALITY CONTROL

This section outlines safety guidelines to follow when using liquid nitrogen, as well as information about Installation and Operational Qualifications (IQ/OQ) that help you ensure proper equipment installation and testing.

LIQUID NITROGEN SAFETY

The low temperature storage of biological materials presents significant safety hazards. A little common sense goes a long way when handling liquid nitrogen, which displaces oxygen during evaporation. Nitrogen gas is colorless, odorless, and tasteless. It cannot be detected by the human senses and is breathed as if it were air. Breathing an atmosphere that contains less than 18% oxygen can cause dizziness and quickly lead to unconsciousness and death.

EXPLOSION

Liquid nitrogen is a cryogen with a boiling point of -196C (-320F). When removed from a liquid nitrogen atmosphere, improperly sealed sample containers may explode. Placing containers in vapor phase nitrogen for several hours before immersing them in liquid nitrogen minimizes the risk of explosion.

CONTAMINATION

If the container being immersed in liquid nitrogen contains hazardous biological materials, the container should be thawed and opened in a biological safety cabinet. Broken containers within storage freezers may be a risk due to contaminant survival. Protocols for decontamination should be developed for immediate corrective action if contamination occurs.

PROTECTIVE CLOTHING

A protective face covering, insulated gloves, and long sleeved clothing help prevent unnecessary exposure to liquid nitrogen.

SAFETY RECOMMENDATIONS

Always consider the following when working with liquid nitrogen:

- Use only containers designed for low temperature liquids.
- Do not seal or prevent liquid nitrogen from venting.
- Use solid (never hollow) rods as measurement sticks.
- Use liquid nitrogen in well ventilated areas only.
- Do not overfill containers.

IQ/OQ PROCEDURES

Reasons to validate processes include improving customer satisfaction, reducing costs, improving product quality, and fulfilling regulatory requirements. A validated process, coupled with process design control can reduce development time, which could lead to a faster time to market for a product. A properly validated and controlled installation process will yield a better performing product and is likely to result in fewer complaints and recalls.

Methods and procedures for developing Installation and Operational Qualifications vary from company to company. While some customers follow specific formats for equipment in their institution, many manufacturers make IQ/OQ procedures available with their individual equipment as a point of customer service. IQ/OQ procedures are a vailable for Thermo Electron's CryoMed Freezers and Cryo/CryoPlus LN_2 Storage Systems. These procedures, which meet the requirements of ISO 9001, include fully detailed checklists to qualify equipment setup and use. A document disk is included for easy process customization.

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Installation Qualification

Simply put, IQ answers the question "Is it installed correctly?" Important considerations in determining this qualification are as follows:

- Equipment design features (construction, cleanability, etc.)
- Installation conditions (wiring, utilities, functionality, etc.)
- Calibration, preventive maintenance, and cleaning schedules
- Safety features
- Supplier documentation, prints, drawings, and manuals
- Software documentation
- Spare parts list
- Environmental conditions (ambient temperature, exhaust requirements/oxygen supply)

Sometimes quality control activities are conducted at the manufacturer's site before equipment is shipped. For example, equipment manufacturers may perform test runs at their facilities and analyze the results to determine if the equipment is ready for delivery. These studies can be used as guides to obtain basic data and to supplement your installation qualification.

Operational Qualification

In the OQ phase, process parameters are challenged to assure that they will result in a product that meets defined requirements under anticipated manufacturing conditions. Considerations include the following:

- Process control limits (time, temperature, pressure, linespeed, setup conditions, etc.)
- Software
- Raw material specifications
- Operating procedures
- Control procedures
- Preventive maintenance
- Training
- Cleaning
- Calibration
- Evaluations for potential failure modes and worst case conditions

An Installation Qualification helps ensure that your equipment is installed safely and properly. An Operational Qualification addresses equipment use and maintenance.

Another type of qualification procedure—a Performance Qualification (PQ)—is specific to an individual's or an organization's work. Typically, PQ procedures are not provided by equipment manufacturers.

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