



TECHNICAL BULLETIN

Cryopreservation Basics

Cryopreservation - Semi-Permanent Preservation

The only effective method of preserving animal cell cultures is by freezing, which can be accomplished with either liquid nitrogen or cryogenic freezers. The freezing process involves slowly reducing the temperature of prepared cells to -30 to -60°C and then transferring to temperatures less than -130°C. Once at ultralow temperatures, the cells are biologically inert and can be preserved for years.

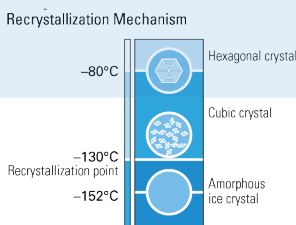
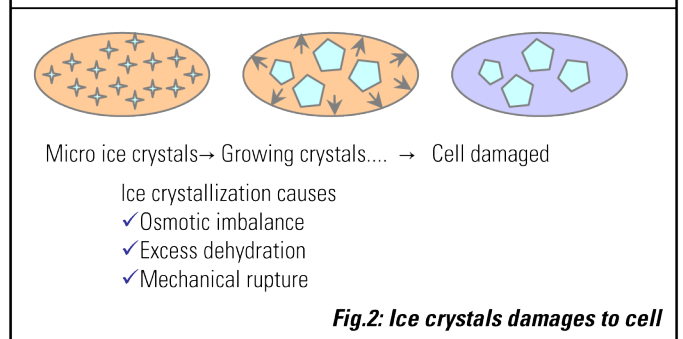
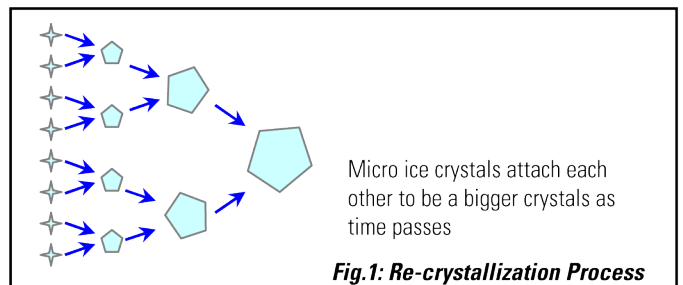
Cryopreservation is the storage of biological materials at ultra low temperatures. The benefit of this is the level of freezing damage to cells is minimized. When preserving cells or tissue at cryogenic temperatures, the main challenge is to prevent amorphous ice crystals from recrystallizing within and outside the cells. These ice crystals increase the degree of freezing damage to cells. When ice crystals become amorphous, they are stored using cryoprotective agents such as glycerine and dimethyl sulfoxide (Me2SO). The speed of ice crystal formation is restricted during preservation below a certain temperature when complete vitrification is possible. -130°C is the recrystallization point of pure water and the ultra-low temperature at which amorphous ice crystals recrystallize.

The problem: Ice re-crystallization creates freezing damage

Biological materials consist mostly of water. During the freezing process, free water inside and outside the cells change to ice and cellular metabolism ceases. To achieve effective long term preservation with high viability maintained, it is crucial to prevent the movement of ice crystals. To cause further damage, micro ice crystals attach to each other and grow bigger as time passes (Fig. 1). The larger crystals chemically and physically damage cells, which result in compromised samples (Fig.2) after thawing.

Vitrification –the glass state

Vitrification is a process of converting a material into a glass-like amorphous solid free from any crystalline structure, either by the quick removal or addition of heat or mixing with an additive. When samples are stored under a certain temperature, the water in the system converts to this glass-like state. The glass state is a scientific phenomenon- liquid in a molecular basis (also called the 4th state), but that behaves like a solid because of the high viscosity. This glass state is quite stable and free of freezing damage.

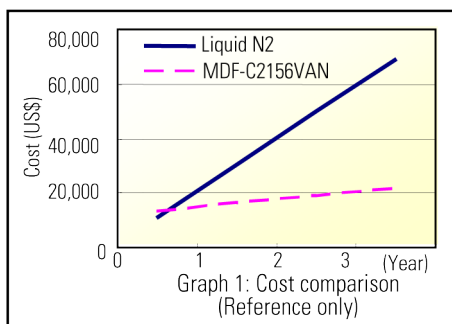


Preservation under the glass transition temperature

For pure water, the glass transition temperature is about -130°C . By adding cryoprotective agents such as glycerin or DMSO to mediums, the glass transition temperature can rise even higher, to -115°C . However, some agents have toxicity with limited use, making it is necessary to store samples at the lowest temperature possible to achieve long-term storage with high viability. Cryogenic preservation allows for the semi-permanent storage of biological materials with a high survival rate.

The -150°C Mechanical Freezer's Advantage over Liquid Nitrogen

Liquid nitrogen storage has been the prevalent scientific solution for cryopreservation. However, LN_2 tends to vaporize continuously since its boiling point is -196°C . It also requires a periodic LN_2 supply replenishment for the storage system. The LN_2 storage container is very expensive and running costs are a continuous burden. SANYO is one of the very few manufacturers providing -150°C mechanical freezers for an alternate method of cryogenic storage. The SANYO -150°C freezer is much more cost effective since it does not require the continual maintenance costs of liquid nitrogen. (Graph 1).



Key Points

- Cryogenic mechanical freezers are an alternative to the traditional methods of preserving animal cells
- Cryogenic freezers use high efficiency compressors to reach temperatures as low as -150°C
- No filling of LN_2 is necessary, but LN_2 back-up systems are available for added security in MDF-C2156VANC
- Freezers are generally easier to catalog than many liquid nitrogen systems

THE SANYO ADVANTAGE (An Ideal Alternative to LN_2 Storage):

- Lower running cost
- Easy maintenance
- Improved Safety and Usability
- Innovative Control with LCD panel
- Reduced Cell Cross Contamination
- Vapor State Storage Temperatures
- Increased Long Term Cell Preservation

Competitive Comparison

	SANYO MDF-C2156VANC	Competitor 'R'
Capacity (Liter/Cu.ft.)	231L/8.2 cu.ft.	193L/6.8 cu.ft.
2" Box Storage Capacity	150	90
Inner Dimension (mm)	760 x 495 x 615	597 x 483 x 673
Outer Dimension (mm)	1730 x 765 x 1010	1452 x 737 x 1086

Product Benefits: MDF-C2156VANC Cryogenic Freezer

1. Improved functions for safety, storage and security
 - New double door gasket prevents heat leakage
 - Enhanced thermal insulating by new VIP® Plus technology
 - Warning notice of failure prediction through an additional status function
 - Ability to monitor freezer status by a log function

- Controlled access to valuable or hazardous samples (plus an optional digital lock)

2. Environmentally-friendly freezer

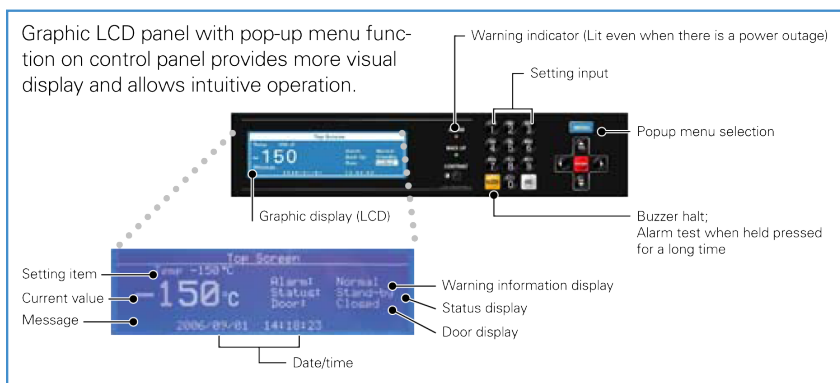
- 2-stage mixed refrigerant system with NON-HCFC refrigerant
- Reduced power consumption
- New VIP® (Vacuum Insulation Panel) Plus - SANYO Breakthrough Technology

Applications:

- Cancer research: Tumor Cells
- Blood Banking: HLA, Red Cells
- Bone Marrow Preservation: Skin, Bone, Heart Valves, Corneas, Musculoskeletal Tissues
- Microbiology: Viruses, Bacteria, Cell Culture
- Genetic Engineering – Cultures
- HLA: (Lymphocytes) Organ Transplant
- IVF: (Embryo, Sperm) Human Infertility
- Sperm & Fertilized Ovum (Bull, Goat, Horse, Pig, Chicken) Preservation
- Plant Cell Preservation
- Monoclonal Antibody Preservation
- Pharmaceutical: Cultures
- Repositories: Cell Culture

Targets

- 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



-150°C Mechanical Freezer and Liquid Nitrogen Storage System Comparison

	SANYO -150C Mechanical Freezer		Liquid Nitrogen Storage System	
Cooling temperature	-150C at 30C with good uniformity	☺	Vapor phase: -180C to 190C Liquid phase: -196C (Some cryo-tubes are only usable only for the vapor phase)	☺
Reliability	Built-in LN2 back-up (standard for MDF-2156) and a broad alarm system equipped	☺	Periodic residual LN2 monitor is necessary	☹
Operation cost	Initial cost and running electricity cost (See Graph1)	☺	Initial cost, continuous LN2 supply cost and small electricity cost for a monitoring device, etc. (See Graph1)	☹
Maintenance manpower	Less maintenance duties	☺	Periodic LN2 supply and routine residual LN2 monitoring is needed. An oxygen deficiency risk exists when opening the container (sometimes deadly accident occurs)	☹
Usable space	100% of interior space usable	☺	Interior space is partially unusable because of the narrow mouth shape that prevents LN2 vaporization.	☹
Contamination risk	None	☺	Cross-contamination and mycoplasma contamination risks in liquid phase storage	☹
Benefits	Convenient operation, easy to place and retrieve samples, superior cost performance, large capacity and no contamination risk	☺	The lowest temperature storage in current storage systems. Various sizes and portable systems available	☺



Preservation