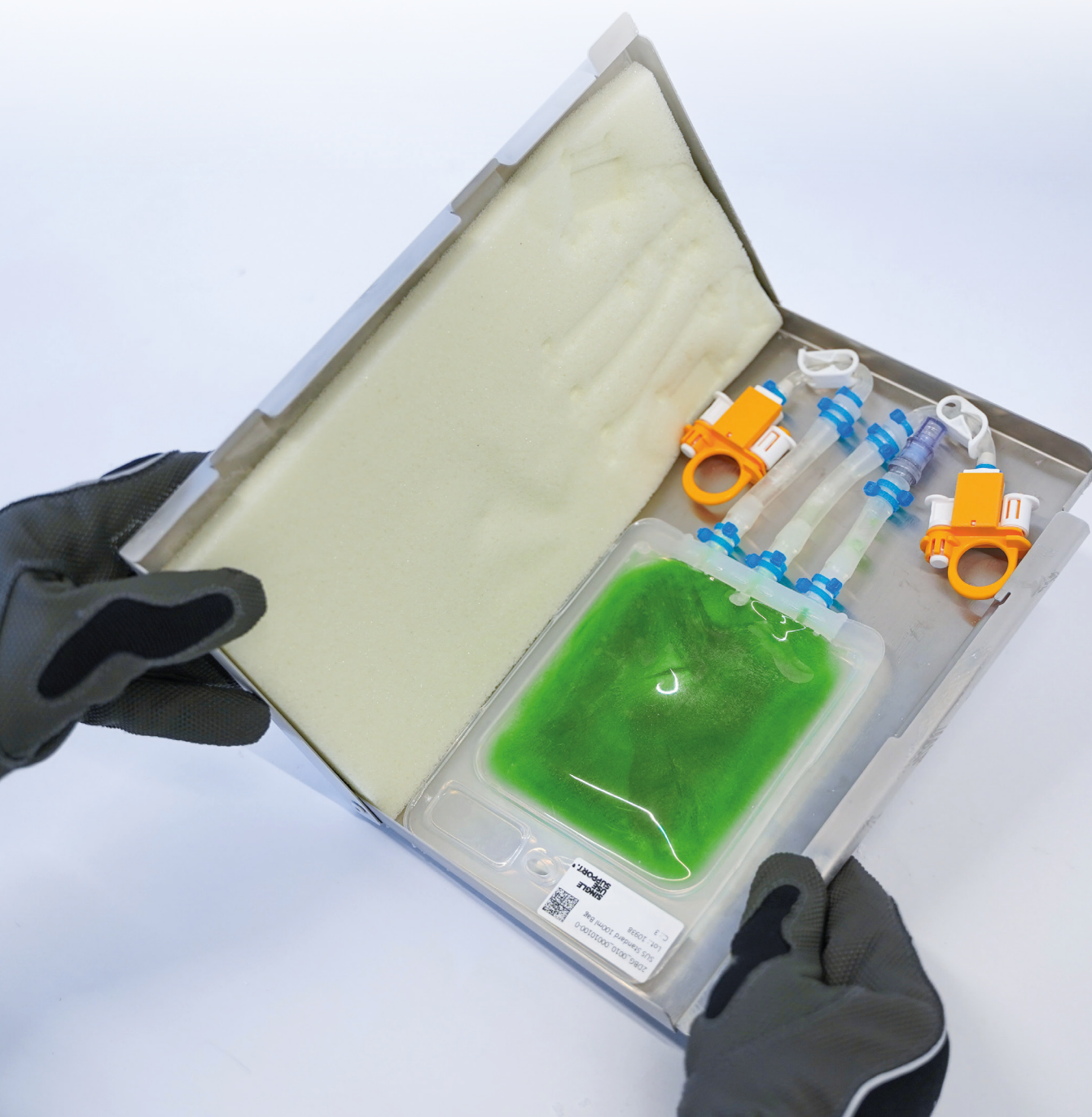


**SINGLE
USE
SUPPORT.** 
PIONEERING BIOPHARMA



READY FOR THE COLD? ATMP CRYOPRESERVATION DONE RIGHT

Best practice in small volume cryo-freezing





Introduction

As the biopharmaceutical landscape sees a rising demand for cell and gene therapies, manufacturers are faced with increasing pressure to meet those needs, which is why manufacturing equipment must follow suit and support the optimization of drug development and manufacturing processes.

The industry emphasizes speed and cost efficiency in fluid and cold chain management processes for small-volume Advanced Therapy Medicinal Products (ATMP). Given that cells are typically preserved at ultra-low temperatures (ULT), maintaining sterility throughout the entire cell and gene therapies (CGT) manufacturing process is crucial. This applies to fluid transfer into small single-use bioprocess containers and cryogenic freezing of sterile single-use consumables. Reducing complications and risks remains a top priority across the product lifecycle, from clinical research to commercialization.

Challenges by manufacturers

The quest to find the best equipment for a CGT manufacturing process is undoubtedly riddled with many challenges. The top 3 challenges manufacturers are looking at when working with cells are:

- **Leakages:** When cryopreserving cells at ultra-low temperatures, as low as $-190^{\circ}\text{C}/-310^{\circ}\text{F}$, it is crucial to protect single-use bags, their assemblies, and aseptic connectors from contamination caused by material failures such as leakage or bag breakage.
- **Cell death:** Freezing cells poses a natural threat to cell viability. The addition of DMSO and uncontrolled freezing can further reduce cell viability during a freeze-thaw cycle.¹
- **Inefficiencies:** Tube welding is a manual approach to maintaining a sterile fluid path, requiring operator resources and slowing down the manufacturing process. Other cost drivers include ineffective fluid handling, like manual filling, which leads to defects and product loss.²

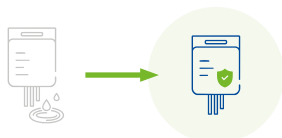


1. Essani K.: DMSO: Freezing Cells. 2024. Single Use Support. Available at: DMSO: Freezing Cells

2. Sherrick A., Pendlebury D.: Gene Therapies: A Guide to Aseptic Single-Use Connectors. 2021. Colder Products Company. Available at: <https://www.cpcworldwide.com/Resources-Support/Literature/Biopharma/Gene-Therapies-Guide-to-Aseptic-Single-use-connectors>



What can be done to address those problems?



1. Safeguarding integrity during cryopreservation

All elements of a CGT single-use system must maintain integrity. These include the primary packaging, such as single-use bags and assemblies, and sterile connectors. Particularly during cryopreservation of cell and gene therapies, all these components must withstand cryogenic temperatures to reduce the risk of product loss through leakages and the associated risk of contamination.³

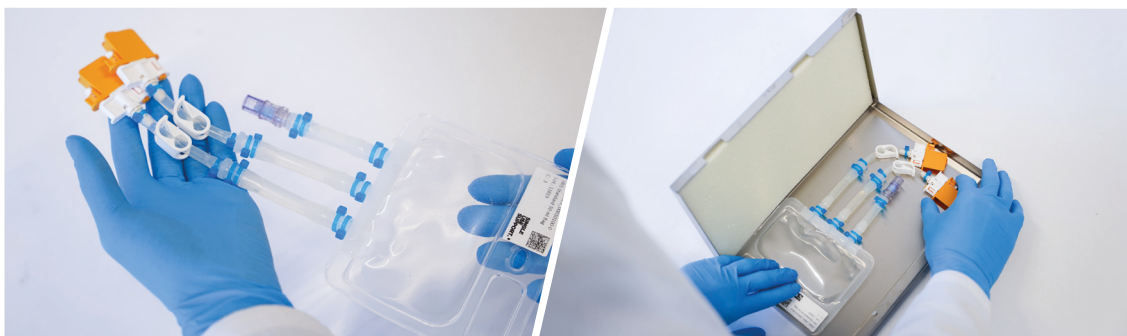
How cold can it get?

Cells are typically frozen to cryogenic temperatures between -130°C/-202°F and -190°C/-310°F.⁴ Therefore, all components must withstand this cold during cryogenic freezing and storage to maintain integrity and functionality. The freezing package usually consists of:

- Single-use bag
- Single-use tubing assembly
- Aseptic connector
- Secondary packaging

CPC's MicroCNX® ULT and Nano are aseptic connectors that can be attached to all single-use bags through 1/8" and 1/16" assemblies, with the ULT also being compatible with 3/32" tubing size. The connectors link two fluid pathways while maintaining sterility. Each connector half features a sterile barrier that prevents bacteria and contaminants from entering the fluid pathways when the barrier is in place. The MicroCNX® ULT and Nano series are the only aseptic micro-connectors that can be frozen with cryopreservation bags placed directly into a protective freezing shell, streamlining material handling. This makes them a safe and tamper-evident aseptically closed system.

Single Use Support's RoSS.KSET is a protective secondary packaging solution designed to accommodate all available types of single-use bags up to 250mL, single-use assemblies and MicroCNX® ULT and Nano during freezing, storage and shipping at ultra-low temperatures. The combination of the protective cassette and its foam inlays immobilizes the single-use bags and assemblies, minimizing the risk of product loss due to leakage to 0%.⁵

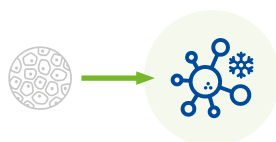


50mL IRIS single-use bag with MicroCNX® Nano connectors in RoSS.KSET system

3. Seifert D.: Navigating GMP Annex 1: How single-use assemblies will comply. 2023. Single Use Support. Available at: <https://www.susupport.com/knowledge/single-use-consumables/navigating-annex-single-assemblies-will-comply>

4. Fuchs A.: Pharmaceutical cold chain management – overview of all freezing ranges. 2022. Single Use Support. Available at: <https://www.susupport.com/knowledge/freeze-thaw/pharmaceutical-cold-chain-management-overview-freezing-ranges>

5. Exenberger C.: Case Study: Reducing Product Loss | Cost-efficiency. 2022. Single Use Support. Available at: <https://www.susupport.com/knowledge/single-use-consumables/case-study-reducing-product-loss-cost-efficiency>



2. Maintaining cell viability

Whether freezing cells, nucleic acids or proteins, one goal applies to all of them: achieving the highest possible product quality during freeze-thaw cycles. This will help manufacturers improve productivity and efficiency. Let's take a closer look at how cell viability can be maintained during cryopreservation:

Controlled freezing

Controlled-rate freezing technologies help improve product quality. A study shows that the viability of mammalian cell lines is best when they are frozen at a controlled rate of $-1^{\circ}\text{C}/\text{min}$ to $-4^{\circ}\text{C}/\text{min}$ in accordance with freezing protocols. These protocols ensure a steady temperature decrease while accounting for the critical phase transition. RoSS.KSET enables cell-based therapies to be frozen using controlled-rate plate freezers, or controlled-rate blast freezers. With the cooling medium and the bag only separated by a stainless-steel lid to provide the best heat transfer, this is an efficient way to standardize scalable freezing of cell-based products such as CHO cells.⁶



Controlled-rate freezing of small single-use bags with Single Use Support's plate freezer and LN2 freezer

DMSO compatibility

To reduce cryopreservation-induced damage even further, cryoprotective agents (CPAs) such as DMSO (dimethyl sulfoxide) and glycerol are usually added to cell-based products prior to being subjected to a freeze-thaw cycle.¹ All components of the primary containment system must be chemically compatible with these CPAs, which include single-use bags, assemblies and aseptic connectors.

To meet the evolving needs of cell and gene therapy, CPC's innovative MicroCNX[®] series connectors are designed to withstand temperatures down to $-190^{\circ}\text{C}/-310^{\circ}\text{F}$ and are chemically compatible with cryoprotective agents such as DMSO.² When cryoprotectants are added to freezing cells, the MicroCNX[®] ULT and Nano are validated for integrity with up to 75% DMSO.² The DMSO integrity of single-use bags and assemblies depends on the validation of their films, as well as the extrac-tables and leachables data.

1. Essani K.: DMSO: Freezing Cells. 2024. Single Use Support. Available at: DMSO: Freezing Cells

2. Sherrick A., Pendlebury D.: Gene Therapies: A Guide to Aseptic Single-Use Connectors. 2021. Colder Products Company. Available at: <https://www.cpcworldwide.com/Resources-Support/Literature/Biopharma/Gene-Therapies-Guide-to-Aseptic-Single-use-connectors>

6 Brandmayr P.: Optimization of the freezing process with a liquid nitrogen freezer to increase cell viability of a mammalian cell line. 2023.



3. How to make sterile connections faster and safer?

Efficiency | Speed

Sterile connectors facilitate CGT processes outside of the biosafety cabinet (BSC), replacing the need for tube welding. From a productivity standpoint, an experienced operator can establish an aseptic connection with a sterile connector in less than 10 seconds, versus the estimated 3-7 minutes required for creating a tube weld.

A production environment making 100 connections via tube welding in a week will require about 5 to 11 hours of operator time. The time required varies based on factors such as the tube welder, operator skill level, replacing blades and other potential delays. In contrast, an operator using sterile connectors could make 100 sterile connections in less than 17 minutes.²



Instruction for use of MicroCNX® Nano to connect fluid pathways

Efficiency | Cost savings

Breakage of single-use bags or assemblies means loss of very valuable biopharmaceuticals; robust secondary packaging for freezing, cold storage and shipping helps improve bag integrity and standardization of bags.

A global biotechnology company producing monoclonal antibodies (mABs) was experiencing product loss rates between 0.5% and 5% due to the sensitivity of single-use bioprocess containers at sub-zero temperatures. After implementing Single Use Support's RoSS® secondary packaging for bags for a batch size of hundreds of liters, there was **not a single bag break or other product loss**.⁵

Improved productivity has made the manufacturing process more efficient by increasing the yield per batch produced: less product loss means more available resources.

95%
SAVED TIME
with MicroCNX²

99%
REDUCTION IN PRODUCT LOSS
with RoSS® shell⁵

2. Sherrick A., Pendlebury D.: Gene Therapies: A Guide to Aseptic Single-Use Connectors, 2021. Colder Products Company. Available at: <https://www.cpcworldwide.com/Resources-Support/Literature/Biopharma/Gene-Therapies-Guide-to-Aseptic-Single-use-connectors>
5. Exenberger C.: Case Study: Reducing Product Loss | Cost-efficiency, 2022. Single Use Support. Available at: <https://www.susupport.com/knowledge/single-use-consumables/case-study-reducing-product-loss-cost-efficiency>



Best practice: MicroCNX® & IRIS bags in RoSS.KSET

The two vendor-independent product innovations, CPC’s MicroCNX® ULT and Nano and Single Use Support’s IRIS single-use bags protected by RoSS.KSET, together form the best practice for small volume cryopreservation and cryogenic applications of ATMPs.

MicroCNX® Aseptic connectors for CGT

- Available in 3 sizes
 - ULT in 1/8“, 1/16“ and 3/32“
 - Nano in 1/8“ and 1/16“
 - ULT will fit with most and Nano will fit with all commercially available freeze cassettes
- The only aseptic micro-connectors available for CGT manufacturing processes
- Validated for cryogenic applications down to -190°C/-310°F
- DMSO compatible



MicroCNX® <i>Series Connectors</i>	Profile Height	Tubing Size			Tubing Material			Cryo Compatibility	
		1/8"	1/16"	3/32"	TPE	Silicone	PVC	Freeze to -190°C/-310°F	DMSO
MicroCNX <i>ULT</i>	Std: 0.62" Metric: 15.7mm 	✓	✓	✓	✓	✓	✓	✓	✓
MicroCNX <i>Nano</i>	Std: 0.31" Metric: 7.9mm 	✓	✓		✓	✓	✓	✓	✓



RoSS.KSET Protective freezing cassette for small single-use bags

- Protection of IRIS single-use bags and all other available tubing, connectors and bag types
- Available in 3 sizes to accommodate single-use bags of up to 250mL
- Validated for cryogenic applications down to -190°C/-310°F
- Enables controlled-rate plate freezing
- Reduces product loss to < 0.001%



Benefits



Vendor-agnostic

No limits with the best-practice setup for cell and gene therapy freezing

- MicroCNX® and RoSS.KSET can support single-use bags from any manufacturer
- MicroCNX® aseptic connectors fit into all freezing cassettes
- RoSS.KSET can accommodate all single-use bags and aseptic connectors



Closed & safe

Aseptically closed system safe from contamination

- MicroCNX® and cryopreservation bags can be placed into and frozen in RoSS.KSET protective freezing cassette
- RoSS.KSET protects single-use systems to reduce product loss due to bag breakage



Simply efficient

Fast aseptic connections for CGT processes

- Save time and resources compared to welding & biosafety cabinets
- Increase production yield thanks to lower failure rates

More about Single Use Support



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More about CPC



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