SINGLE USE SUPPORT.

PIONEERING BIOPHARMA

ADVANCING ATMP FLUID MANAGEMENT

HOW TO STREAMLINE COMPLEX SMALL VOLUME PROCESS LOGISTICS

WHAT ARE ATMPS?

"Advanced therapy medicinal products (ATMPs) are medicines for human use that are based on genes, tissues or cells. They offer ground-breaking new opportunities for the treatment of disease and injury"

EMA, European Medicines Agency

1.1 BACKGROUND

Advanced Therapy Medicinal Products (ATMPs) comprise of three classes[1]

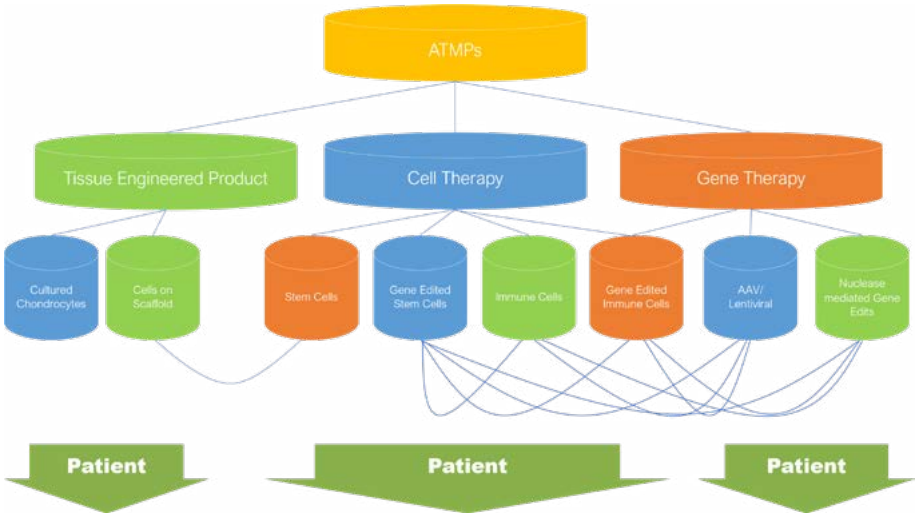
Gene Therapy
A faulty or missing gene is replaced or silenced

Cell Therapy
Cells are removed from a donor and manipulated before being infused into a patient

Tissue engineered product
Cells or tissue samples are removed from a donor and engineered or adapted in the lab to form functional tissues and then administered to the patient

Cell and Gene Therapy comprises of different approches to manipulate and boost the immune system to fight against deadly diseases such as cancer. The success potential in oncology, hematology, immunology, the treatment of rare diseases and others is enormous and has kicked off a global drive for such therapies. There is a lot of crossover in the field of advanced therapies: For example, adeno-associated viral vectors and lentiviral vectors are used in gene therapy, but also in gene-edited stem cells.

ATMP Overview[1]



1.2 STATUS QUO

There is a growing market for cellular and gene therapies and more and more therapies are being studied. There a numerous ongoing, FDA- and EMA-approved, therapies with only few of them being marketed. Both autologous and allogeneic cell therapies are expected to play a big role in the future of advanced therapies.

Allogeneic cellular therapies in particular tend to have larger volume ranges, whereas autologous therapies tend to remain small volumes. Overall, the volumes of cell and gene therapies are ranging from several mL to multiple liters. Scalable fluid management solutions are key to providing consistent handling throughout different scales and to safeguarding reproducibility and consequently patient safety.[2]

Cell Therapy Landscape[3]

1.3 ASEPTIC ALIQUOTING OF ATMPS

Bioprocessing is still dominated by non-closed, in-house solutions, with high levels of manual intervention that drive process inconsistency and increase the risk of contamination. The major technology gaps are filled by automated aseptic dispensing platforms that transfer cell and gene therapies from one process step to another. Automated solutions solve challenges in a wider range of applications to meet the needs of the biopharmaceutical market, such as the following:

Seed Train Intensification: The fully automated aliquotation of cells into smaller single-use bags helps establish a process to enhance productivity in seed train intensification including aliquoting, homogenizing and freezing cells for maximum cell viability. High density cells to inoculate the N-stage production bioreactor are dispensed in aliquots ranging from 30mL up to 1L.

Media Preparation: The aliquotation of media into single-use bags is part of downstream bioprocessing. Highest filling accuracy ensures that the correct volumes are aligned with the next process step. Fast fill & filtration is possible up to 400L per batch.

Fill & Finish Small Volumes: In the fill & finish process step, small volumes that are used in a subsequent process step to make a gene modified cell therapy have to be filled into primary packaging. Small volume filling of down to 3mL makes it possible to switch from vials to bags, which requires the highest degree of precision.

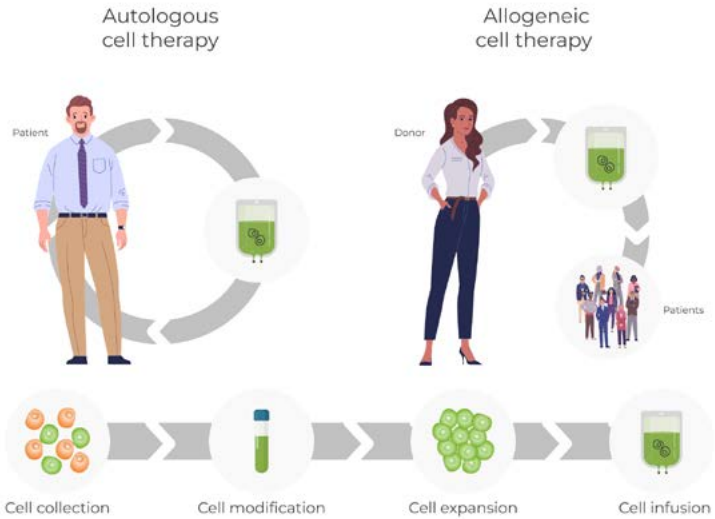

RoSS.FILL CGT


RoSS.FILL Base


RoSS.FILL Bag


RoSS.FILL CGT Small Volume

Autologous vs. Allogeneic Cell Therapy[2]



1.4 FREEZING & THAWING OF ATMPs

Whether in the early stages of drug discovery or during the commercial production of small volumes, controlled freezing of ATMPs becomes an essential element in safeguarding the quality of drug products throughout the manufacturing journey.

Several scenarios highlight the relevance of controlled freezing of small volumes[4]:

Working cell banks (WCB): Cell banks are required for many different areas of applications. Mammalian CHO cells are required for different cell-based therapies in biomanufacturing. These can be autologous cell therapies, such as CAR-T, or allogeneic cell therapies, emphasizing the need for meticulous control in the freezing process to ensure optimal cell viability.

Cryopreserved HEK293 cells for viral vectors: HEK293 cells are used as host cells for viral vectors – applied in gene therapies. The controlled freezing of these cells is vital to maintain their integrity and functionality and ensure the success of gene therapy applications.

Non-viral vectors in vaccine production: Vaccine production involves the use of non-viral vectors such as lipid nanoparticles and plasmids. Controlled freezing of these components is essential to preserve their efficacy, contributing to the overall success of vaccine manufacturing.

Cell banking in seed train intensification: Employing controlled freezing in seed train intensification is a strategic approach to enhance bioprocess productivity and efficiency. The optimization of cell banking processes, with a special focus on achieving high cell density, is a decisive aspect for success in advancing upstream bioprocessing.

2 CHALLENGES IN ATMP MANUFACTURING

2.1 PROCESS VOLATILITY

Commercial manufacturing of advanced therapies is filled with complexities caused by individual requirements and different product characteristics. Therefore, manufacturing of ATMPs will always require a certain level of process flexibility to balance out changing parameters such as scale-out or scale-up of volumes. CDMOs face challenges when producing several different drug products, each having different batch sizes or primary packaging.

Cell and gene therapies as well as tissue-engineered products usually require small volumes of autologous matter. Along with the need for substantial manipulation, the inability to scale is driving costs in the field of regenerative medicine. This is why manufacturers are looking to adopt flexible systems and solutions that are capable of quickly adapting to different requirements in terms of filtration, aliquotation volumes, bag sizes, homogenization, or even sealing of single-use assemblies. Only modular systems balance out the process variability.

2.2 RISK OF CROSS-CONTAMINATION

Regenerative medicine requires an environment designed to modify human cells and genetic material. As is the case with biopharmaceutical applications it is of the greatest importance to prevent cross-contamination to safeguard patient safety. A robust risk management strategy in compliance with all standards and regulatory considerations, such as current Good Manufacturing Practices (cGMP), is required to minimize the risk of contamination. Refined in cGMP Annex 1, aseptically closed automated systems for fluid management are recommended to reduce the risk of product loss caused by human intervention.

2.3 BIOPROCESSING OPERATION TIME

With patients' lives depending on speedy development and delivery of stem cell- or other autologous therapies, fast delivery is of the essence. Short turnaround times of autologous cell therapies can only be achieved with a functioning and reliable cold chain logistics process utilizing compatible single-use technologies. Downtimes, inefficient handling of liquids or inadequate cryopreservation can result in the loss of personalized medicine. Advanced fluid management processes help make all ATMPs more efficient.

2.4 CHALLENGES OF FREEZING ATMPS

Cryopreservation provides critical protection for cell therapies by minimizing genetic changes. But with a cooling process that is too slow or too fast there is the inherent risk of diminishing cell viability upon thawing. Where blast freezers and liquid nitrogen tanks lack the flexibility needed to adjust freezing conditions according to the specific requirements of a product, the adoption of controlled-rate freezing and thawing methods emerges as a transformative approach in optimizing biopharmaceutical production.[5] The key to successful freezing across diverse applications lies in attaining precise control.

The Power of Controlled-Rate Freezing

One crucial concern in cold chain management, cryoconcentration, can be effectively mitigated through controlled-rate freezing. Cryoconcentration, characterized by the degradation of product quality due to protein aggregation, often results from the slow growth of the ice front in blast and static freezers. This unwanted effect can be averted by implementing controlled-rate freezing techniques.[5]

Addressing individual ATMP characteristics

The evolving interest in understanding a product's behavior during freezing has gained momentum, particularly in determining the cooling rate at which product viability is maximized. Controlled-rate freezing addresses this need, offering tailored solutions, such as plate-based freezing to -80°C/112°F or liquid-nitrogen-based controlled freezing platforms that reach temperatures as low as -170°C/274°F, catering to the specific demands of advanced therapies[6].

Improving Cell Viability

For mammalian cells, optimal freezing rates typically range from -1K/min to -4K/min, reflecting their comfort zone during the freezing process[6]. Recognizing and adhering to the preferred freezing rate is key to maintaining the viability and functionality of these cells.

Do you know your preferred freezing rate?

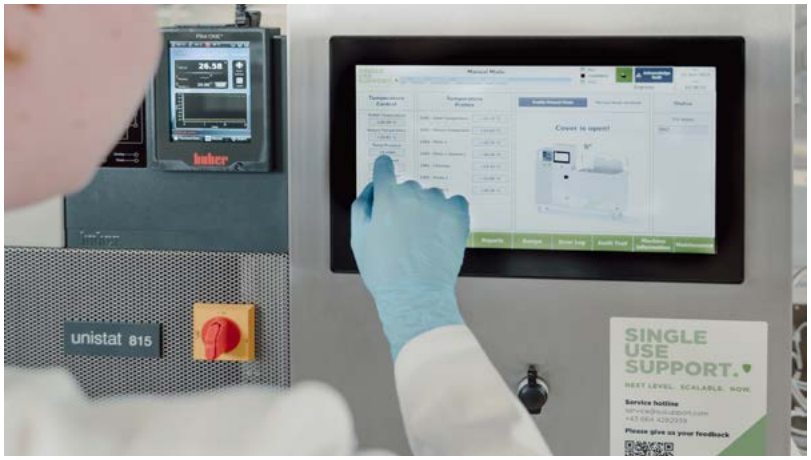
Fighting breakages of single-use systems

When frozen at sub-zero temperatures, single-use bags including tubings and connectors get brittle, which is why they need protection throughout all process steps. RoSS.KSET carries single-use bags of less than 250mL, while RoSS® shell is used for any volume exceeding 250mL. In both cases the robust shell blocks external impact or influence while at the same time giving the single-use bag space to expand during freezing and immobilizing it for safe transportation. In the course of the freezing process, the single-use bag squeezes into the 3D foam inside the protective container. This allows for immobilization and full protection during glass transition, when the bag and its content are at their most vulnerable.

As a closed system, it offers more than just a robust and reliable protection around the single-use bag. Granting consistent product stability and quality across different scales, it fulfills all requirements the industry was waiting for to commercialize single-use technologies in cell and gene therapy. A monitored freeze-thaw process assures optimal product stability in order for high-quality liquids to survive throughout the cold chain.

2.5 SCALABILITY

Instead of blockbusters in vast quantities, the current trend sees the production of small volumes of personalized agents based on mutated cells. And those can easily be generated in small labs instead of giant pharmaceutical plants. The changing requirements in terms of quantities and procedures lead to the need for new and innovative processes that are above all flexible and agile, such as the integration of single-use technology.



In order to not jeopardize a cell therapy's success, a reliable and sterile logistics process with automated steps is crucial – including storage and shipping. Furthermore, some other supplements such as proteins or lysates may also reach bigger batch sizes. Potentially amounting more than 100 liters per batch.

Achieving a consistent freezing output at all scales as well as maintaining ease of use and transferability across different facilities are main challenges in cell and gene therapies. Product efficacy may vary due to differences in freezing performance between laboratory and commercial scale. It is therefore challenging to ensure consistent freezing results across all scales of a given drug substance.

2.6 PROCESS INCOMPATIBILITES

End-to-end solutions for the handling of small volumes enable both process simplification and compatibility throughout then entire drug substance handling process from patient to patient. Ultimately, process solutions, such as those provided by Single Use Support, lead to cost-effective results. The process solutions provider brings together the complementary equipment, technologies, consumables and services for biomanufacturing process solutions. The latter include installation, commissioning, maintenance and performance testing.

With the rapid development of novel regenerative medicine treatments, the need for virus production capacities has increased significantly. Manufacturers are now required to produce a range of highly purified viral preparations in large quantities to address market demand and regulatory constraints.

To do so, they need to build end-to-end, scalable and GMP-compliant manufacturing processes that enable the production of large batches. Such a process is robust, reproducible, scalable and much faster.

This is particularly advantageous for therapeutic companies, for which the availability of GMP-compliant, scalable and transposable processes significantly accelerates clinical trials, mitigating the developmental risks and associated costs.[7]

Only when quality, quantity and ROI are met, industrialization is feasible.

2.7 MANUAL HANDLING

With limited resources, it is important for organizations to ensure they understand the scope and applicability of regulations that impact their automated systems, before focusing their compliance efforts to avoid inefficiencies and over-compliance.[8]

As they have several benefits over manual operations, automated single-use technologies have become an established way to handle medicinal liquids. They offer:

- Flexible use of add-ons (such as filters, connectors, consumables, etc.) as needed
- Limitless scalability (both ways) with single-use bags of any volume
- User-friendly integrability, adaptability and operation
- Standardization and reproducibility of processes
- Complete documentation with automated reports and audit trails

Most importantly, the move towards automated systems will optimize throughput and cost-efficiency while at the same time preventing loss of drug substance by eliminating manual handling steps to rule out the risk of contamination and ensure sterility.

A case study analyzing occurrences at a company handling medium-sized volumes showed that the overall benefits of automated systems are also applicable to plants handling smaller volumes.[9]

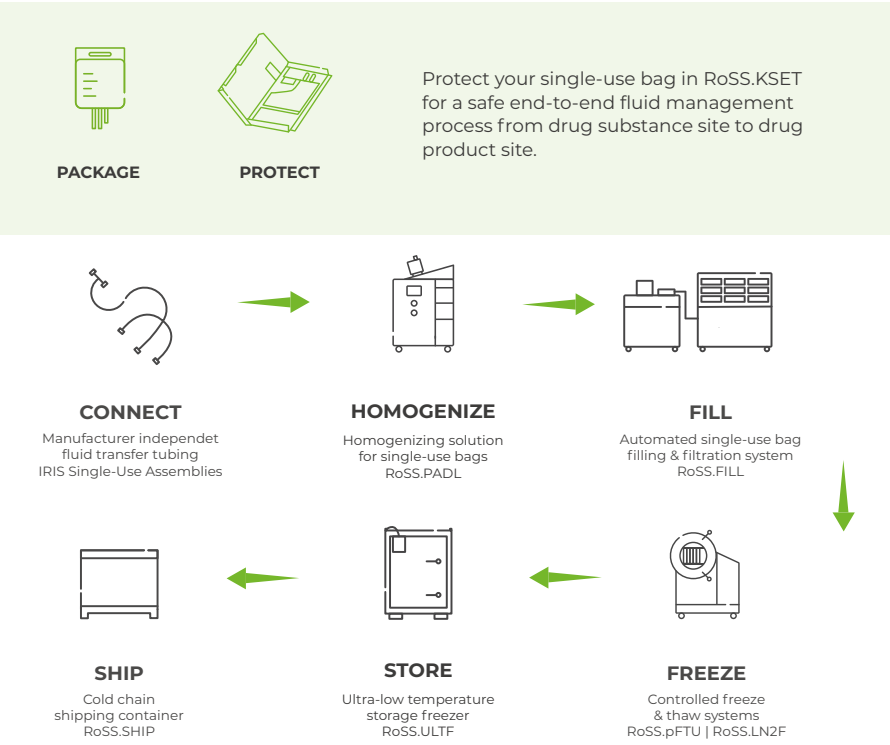
One example: When filling multiple smaller single-use bags (0.1L to 5L), there is a higher occurence of product loss due to residual drug substance in the tubings. By employing technical mechanisms to achieve a satisfactory level of retention, automation makes dispensing in aseptically closed single-use systems an efficient, safe and fast process. "Human error" is the biggest source of faulty operation during filling, which is why automation is crucial in order to minimize product loss as a result of contamination.[10]

3 ATMP FLUID MANAGEMENT

In advanced therapies, the success and reliability of the production process are dictated by the fluid management's precision and efficacy. The challenges involved in handling fluids, be it in transferring, freezing & thawing, storing, or ensuring sterility, are of paramount importance. Addressing these aspects effectively not only impacts the potency and safety of vaccines but also significantly impacts the efficiency and cost-effectiveness of production.

Small volumes in single-use bags

ATMPs in batch volumes ranging from 1mL to 100L are filled into small single-use bags and require universal fill and freeze-thaw solutions for safe handling. Compared to other types of primary packaging, bags are advantageous thanks to their pre-defined freeze path length, reduced contamination risk and simplicity of handling. Moreover, single-use bags facilitate closed systems as well as the utilization of sterile connectors throughout the entire fluid management and freeze/thaw logistics process.



PROTECTING SINGLE-USE BAGS

The secondary packaging, **RoSS® shell**, stands for robust storage and shipping of ATMPs in single-use bags.
The equivalent for small volumes ranging from 1mL to 250mL, **RoSS.KSET**, equally protects bags thanks to its efficient geometric robustness, secure bag immobilization and labeling options that enable reduced product loss and industrial-scale handling for shipping with track & trace.

More benefits:

- Vendor-agnostic: Protection for all available 2D single-use bags from all manufacturers.
- One size fits all: RoSS.KSET is a universal shell for single-use bags of up to 250mL
- Protective shell for cryo applications (-80°C/-176°F)
- Single-use bag covered in 3D foam inlays, protected by robust frame and stainless-steel lids
- Product loss of less than 0.001%
- Freedom and space to use sterile connectors
- Enables advanced plate-based freezing and thawing

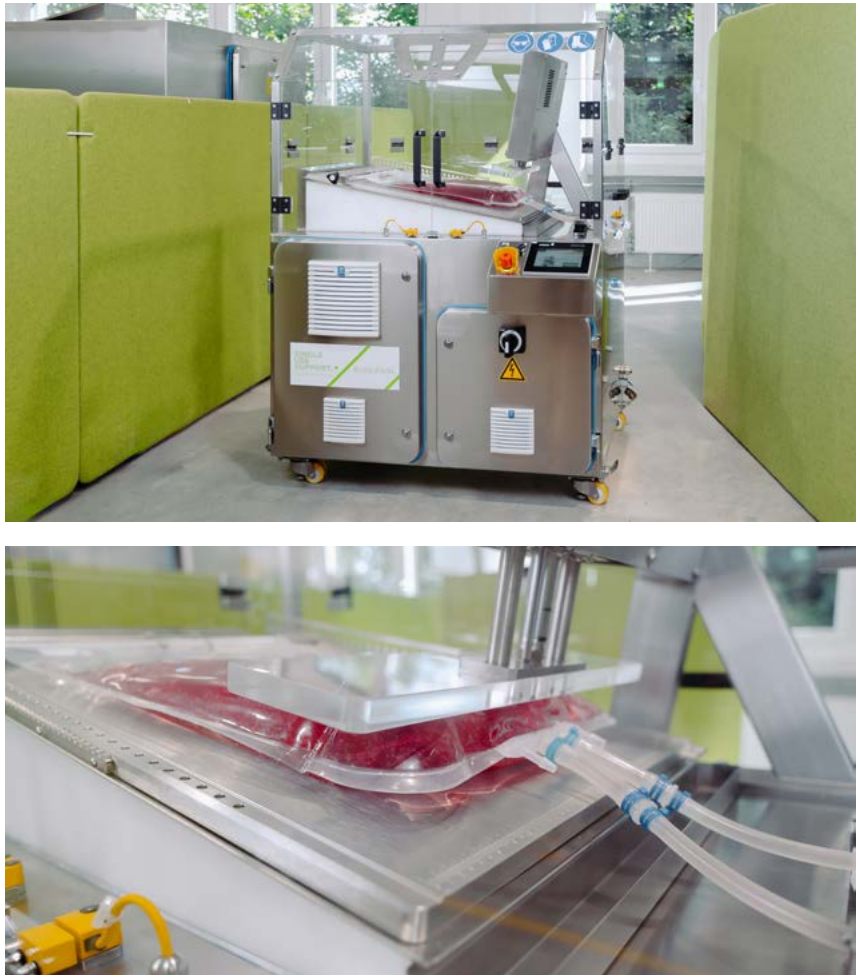
RoSS® shell

HOMOGENIZING

In case an ATMP requires homogenization before aliquotation into single-use bags, **RoSS.PADL** standardizes kneading and cooling of the bioprocess container before dispensing. This enables bag-to-bag consistency.

More benefits:

- Automated process steps enable reproducibility and standardization
- Suitable for all 2D single-use bags of up to 20L
- Bag & connector agnostic
- Bags can be placed side by side with one control system to increase process efficiency
- Seamless integration in end-to-end process solutions



RoSS.PADL

AUTOMATED ALIQUOTATION

Automated aseptic filling of small single-use bags improves productivity to a great extent. **RoSS.FILL CGT** enables consistent, fast, highly accurate, and scalable aliquotation of advanced therapies into bags ranging from 10mL to 500mL. Integrated auto sealing accelerates and facilitates decoupling of bags from single-use assemblies for further process steps.

More benefits:

- Fully automated dispensing system with options for filtration, PUPSIT and draining of single-use bags
- Filling of up to 100+ small single-use bags within approx. one hour
- Expandable to different racks
- Highest possible filling accuracy with gravimetric or flow sensor technology
- Modular system with separate control unit and pumping station
- Possibility to sample

RoSS.FILL

CONTROLLED PLATE FREEZING/THAWING

The plate-based freeze and thaw system **RoSS.pFTU** is capable of freezing at 1°C to 3°C/min (1K to 3K/min) and beyond resulting in optimal product viability, such as cell survival of mammalian cells. The heat transfer through cooling plates enables unprecedented control over the cooling rate to -80°C/-112°F. The rapid, accurate and standardized freezing process is implementable at all scales, resulting in reproducible cryogenic applications independent from the load size per batch. The fully controlled recipe-driven freezing and thawing performance is in accordance with 21 CFR Part 11 and cGMP.

More benefits:

- Single-use bag independent: any bag vendor, any size
- Up to 100L load with different sizes of single-use bags
- Scale-up: Freeze/thaw recipe from smaller platforms are transferable to large scale production
- Scale-out: Best product stability and viability for cells, viral vectors, mABs, pDNA and more
- Fully automated process at highest possible freezing rate and accuracy

RoSS.pFTU

CONTROLLED LN2 FREEZING

RoSS.LN2F is a cryogenic freezer working with controlled exposure of single-use bags protected in RoSS.KSET to liquid nitrogen. With the ability to control cooling rates in the chamber down to -170°C/-274°F, it is the only LN2-based controlled rate blast freezer on the market.

More benefits:

- Freeze your substance of high value in any single-use bioprocess container
- Fully automated & controlled
- Cooling/freezing rates in bags are adjustable from 0.5K/min to max. 4K/min
- Adjustable phase transition time for higher product safety
- Best product stability results

RoSS.LN2F

ULTRA-LOW TEMPERATURE STORAGE FREEZER

Depending on the storage temperature, there are different options for ultra-low temperature storage.

- Storage solutions for final temperatures down to -196°C/-320°F with liquid nitrogen. Transfer of frozen ATMPs at -80°C/-112°F is possible for cryopreservation in LN2 tanks.[6]
- Ultra-low cold storage at -80°C/-112°F with RoSS.ULTF. High storage density paired with a modular interior allows for the storage of a full pallet or primary packaging in customized shelving solutions.

RoSS.ULTF is an ultra-cold freezer and compatible with RoSS® shells to protect your bioprocess containers. It is fully mobile, offers maximum storage space density and its interior can be modularly adapted to individual needs.

More benefits:

- High degree of flexibility in-plant thanks to its portability
- Ease of use for loading: place loaded pallet-sized trolleys or racks inside
- Fully modular: customized shelving systems
- GMP-compliant alarm management

RoSS.ULTF

COLD CHAIN SHIPPING

RoSS.SHIP secures the cold chain for a week during ultra-cold transportation. Get control over your shipment with smart track & trace to never lose control of your product.

Highly robust, stackable, coolable and compact.

More benefits:

- Insulated shipping containers for passive cooling processes
- Qualification based on ISTA / ASTM D4169
- Optional temperature- / GPS- / G-Force tracking
- Adjustable to all requirements and batch sizes
- Suitable for phase-change materials and dry ice
- Single-use or multi-use

RoSS.SHIP

INTERVIEW: REAL-WORLD EXPERTISE

Brian Moloney is Director of New Products and Innovation at Single Use Support. He is leading several projects to develop fluid and cold chain management solutions for advanced therapies.

Q: WHICH BIOMANUFACTURING PAIN POINTS ARE YOU ADDRESSING?

Manufacturers and CDMOs face a multitude of challenges in the evolving fluid and cold chain landscape currently undergoing consolidation. Our mission is to simplify this complexity for customers through modular, compatible, and scalable solutions. This adaptability extends to various production variables, including batch and packaging sizes. Whether dealing with cell-based therapies, viral vectors, plasmid DNA, or other ATMPs, our platform solutions cater to diverse specifications. The added benefit of controlling freezing rates not only eases manufacturing concerns but also contributes to standardized frozen liquid transport solutions. We are dedicated to providing streamlined solutions for evolving production needs.

Q: WHAT'S IMPORTANT WHEN FILLING SMALL VOLUMES?

Ensuring filling accuracy, scalability, process flexibility, and speed is crucial when considering, for example, a cell-based therapy. Achieving consistent cell counts in each sub-100mL single-use bag is a primary concern, necessitating standardized processes for automated aseptic closed systems. These systems must offer operators the flexibility to scale up or out.

Homogenizing and temperature-controlling liquids before filling is essential for maintaining bag-to-bag consistency and high product viability. The clock begins ticking from the moment of exposure to cryopreservatives until reaching the freezing temperature. By chilling the source bag, a broader time window is created for aliquoting the product, optimizing the overall process efficiency.

Q: WHICH BIOPROCESSING STEPS CAN YOUR PROCESS SOLUTIONS BE APPLIED IN?

The beauty of our solutions is that the areas of application can be very wide - from a few mL up to hundreds of liters per batch size. This modular design facilitates effortless scalability for manufacturers. In the advanced therapy domain, our solutions are integral to processes such as seed train intensification, media preparation, cell banking, viral vector manufacturing, and more.

A standout achievement involves aliquoting small volumes down to 3mL, emphasizing the crucial need for precision. We developed an automated cGMP-compliant system capable of cooling, homogenizing, and aliquoting fluid into 128 small single-use bags before automated sealing of the filled single-use bags, ensuring consistent filling accuracy. This innovative approach not only reduced the risk of cross-contamination but also standardized reproducibility, amplifying process efficiency in manufacturing and, most importantly, enhancing patient safety.

Q: WHAT ARE THE BIGGEST MISTAKES ONE COULD MAKE DURING FREEZING ATMP?

I guess the worst-case scenario would be losing a product either to cross-contamination or compromised viability. Without preventative measures, these occurrences can escalate production costs and result in insufficient therapies for patients.

This underscores the significance of our solutions, which prioritize advanced fluid and cold chain management. Our RoSS® shell plays a pivotal role in minimizing bag breakages and cross-contamination, safeguarding the integrity of the single-use bag. Furthermore, it facilitates plate-based freezing, the state-of-the-art method for precisely controlling cooling rates during freezing and thawing, adaptable to product specifications. The direct surface contact between the filled single-use bag and the plate freezer ensures optimal product viability and consistent freezing results, irrespective of batch sizes. This commitment is integral to ensuring a robust and reliable process that minimizes risks and maximizes therapeutic outcomes.

Q: WILL PHARMA 4.0 BE A PART OF ADVANCED THERAPIES?

Certainly, achieving this is feasible, but it entails a step-by-step approach as we navigate the ongoing establishment of fluid and cold chain logistics processes for small volumes. The current industry trend involves a transition from manual to automated processes, paving the way for the integration of digitized solutions in bioprocessing. There is a palpable industry interest in incorporating process steps aligned with Pharma 4.0 principles. As pioneers in biopharmaceutical manufacturing, our commitment is to continually evolve and offer innovative solutions in this domain in the future.

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