CRYOPRESERVATION: Best Practices for Cell-Based Products

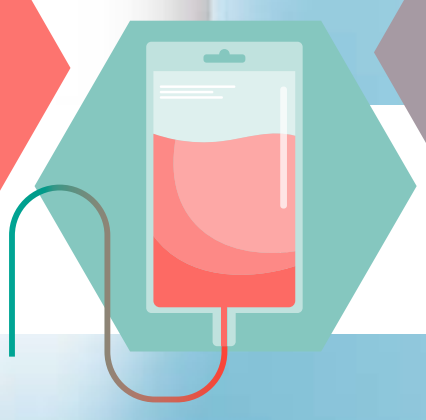
Cell-based products originate from biological starting material, such as cells from tissue biopsies, blood, and bone marrow. These cells can be developed into clinical products *ex vivo*. However, they require specialized processes to remain viable and functional throughout manufacturing, storage, and transport.

Optimizing cryopreservation processes is essential for maximizing product efficacy and process efficiencies. Suboptimal cryopreservation can lower the potency of the final product, as well as greatly increase batch-to-batch variability.

1 Sample isolation & cell processing

Retrieve donor sample at clinical site, package material, and ship to manufacturer with scheduled processing time and set development parameters.

Add a cryoprotectant like HypoThermosol® FRS or CryoStor® CS10 directly to the apheresis material. This may extend sample shelf life and allow for the time it takes the sample to reach the manufacturing facility [1].



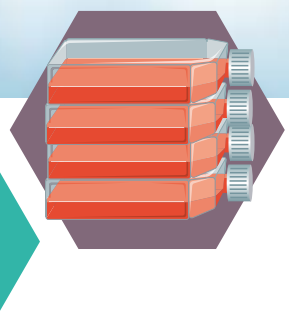
Performance is different for a cryopresered apheresis pack versus fresh. Compare the risks of fresh vs frozen, including post-thaw performance and resource allocation for shipping and manufacturing. How much flex do you have in your schedule, and what does the freeze/thaw process look like at the apheresis centers?

2 Formulation

Perform purification and concentration steps to leave only what will remain in the final delivered drug product. The reduced cell culture is suspended or formulated in cryopreservation media.

Closed-system formulation can reduce contamination risks. Choose a system that provides process flexibility and can grow according to your requirements.

Select a commercially-ready, proven, high-quality media solution, early in development for success at scale. CryoStor® CS10 is the gold standard for cryopreservation. It is supported by extensive scientific evidence, ensures a sustainable supply chain, and will ensure that your production complies with cGMP standards. Good selections made early empower scaled success.



3 Fill

Aliquot formulated product and fill primary containers. This can be done with different commercially-available fluid transfer devices; either automated or manually.

Cell viability clock starts once cryopreservation media is added. Consider how an automated, or semi-automated filling system may facilitate this step compared with a manual approach. Qualify and validate this process against each cell type because holding time will vary.

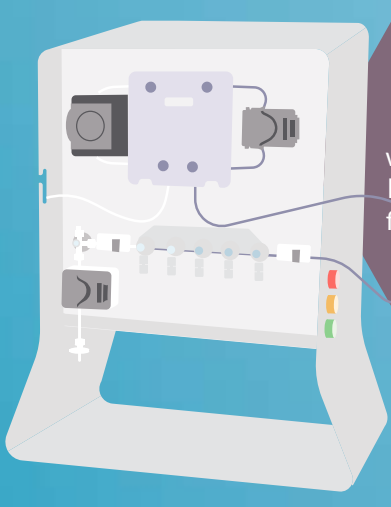
The fill steps demand speed, accuracy and precision. Maintaining solution agitation throughout this step will help ensure homogeneity and avoid sinking cells or coagulation.

When selecting primary containers, consider that your selection meets performance, quality and regulatory requirements. Review for container enclosure integrity, extractables and leachables profile, protection against particulates, and system flexibility for scaling up downstream processes.

4 Freezing the sample

Utilize a controlled-rate freeze to apply a 'seeding' or ice-nucleation step to the sample. This should reduce the risk of a supercooling event and improve sample consistency and process reproducibility.

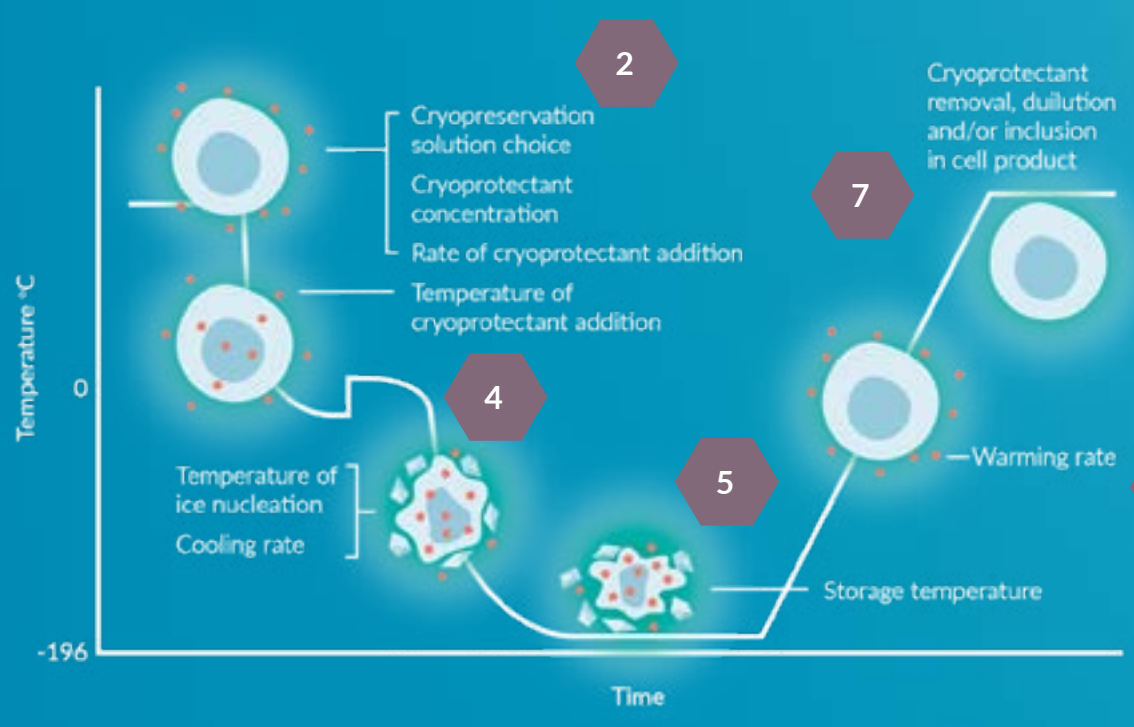
When qualifying the freeze protocol, define the operational space. Understand the smallest and largest payload volume and how it relates to the sample temperature in a certain freeze profile. Vials, bags, cassettes, solution volumes, or even differing container materials will change the freezing profile.



Signata CT-5™ is a flexible, automated and closed fluid-management system capable of formulating and filling drug product. It works with vials, bags or bottles without limiting the number of containers filled in one batch. It incorporates passive cooling and controlled agitation to support product consistency and process efficiency.

Consider how to limit the difference between QC sample containers, retention samples, and final product. Varying materials may change the freeze profile.

Optimize and qualify a cooling rate according to cell type. Use –1°C per minute as a starting point only.



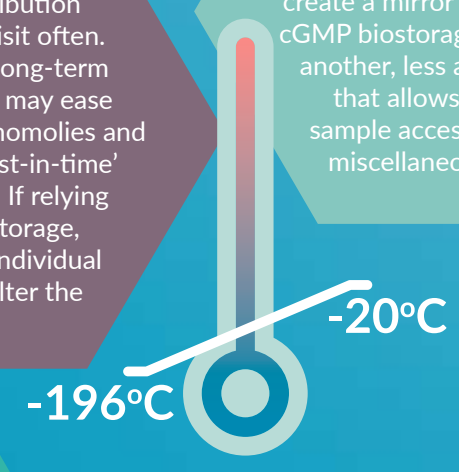
5 Storing the sample

Storage (temperature/humidity) criteria should be defined and qualified to maintain sample integrity and viability throughout the duration of storage and transport.

Partner with a biostorage services company like BioLife Solutions, to meet growing and changing sample storage needs with added cold chain logistic planning and local sample pickup and delivery, free of charge.

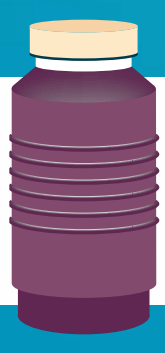
Clinical site storage capabilities vary. Determine distribution plan early and revisit often. Partnering with a long-term biostorage partner may ease clinical site storage anomolies and provide product 'just-in-time' for administration. If relying on clinical site storage, understand how individual site SOPs may alter the product.

If the master cell bank location is prone to natural disasters, (earthquakes, hurricanes, tornadoes, etc.) create a mirror bank with a cGMP biostorage partner in another, less active spot that allows regular sample access without miscellaneous fees.



6 Shipping the sample

Secure transport of biologic material demands shipping solutions with uncompromised thermal integrity and real-time payload visibility.



A shipper such as the evo® DV10 provides up to 15 days of cryogenic protection, with monitored visibility, while limiting handling mistakes.

The manufacturing, distribution, and warehousing strategy needs to be developed early and revisited often. When establishing the plan, consider these questions: How far will the product be shipped from collection to administration? Will a hub-spoke storage and distribution model meet geographical targets? Who is the courier partner to arrange regular cold chain movements? How long will a shipper protect the specific sample type?

7 Thawing the sample

A thawing program must follow a consistent warming algorithm across the sample and be reproducible at any clinical site.

Once the sample has left the manufacturing site, the manufacturer has lost control of the product. Ensure each individual clinic has qualified the appropriate thaw equipment and are amenable to adapting their protocol to meet your sample requirements.

Selecting an automated, mechanical thawing system over a water bath, bead bath, or thermal block limits site variability.

1. Tyagarajan S, Schmitt D, Acker C, Rutjens E. Autologous cryopreserved leukapheresis cellular material for chimeric antigen receptor-T cell manufacture. *Cytotherapy* 2019; 21(12), 1198–1205.
2. Leong L, Narula M, Heslop H, Brenner M, Mamonkin M, Watanabe N. Combining Apoptic Resistance and Cytokine Signaling to Improve Persistence and Anti-Tumor Activity of Vδ2 T Cells *In Vivo* [Poster presentation]. American Society of Hematology, San Diego 2023.

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