teknova: pluristyx®

PluriFreeze™

Cryopreservation System Protocol

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INTRODUCTION

How to Use the PluriFreeze Cryopreservation System

To help you get successful outcomes, our team of cell therapy and reagents experts has put together a proven protocol designed to integrate into your workflow. **Figure 1** illustrates where the PluriFreeze Base and PluriFreeze PF10 reagents fit within a typical cell therapy manufacturing process. If you have any questions about how to design and optimize these steps for your specific application, our team is here to help.

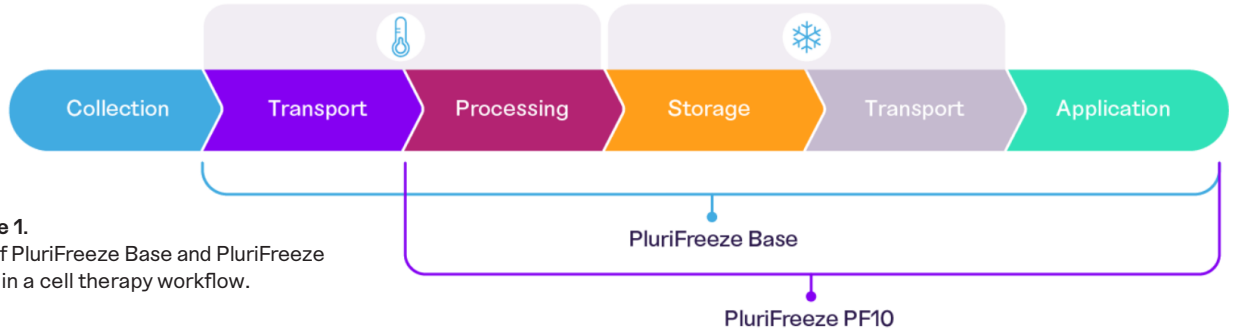


Figure 1.
Use of PluriFreeze Base and PluriFreeze PF10 in a cell therapy workflow.

The PluriFreeze Cryopreservation System consists of two products designed to work together to optimize your cryopreservation outcomes: PluriFreeze Base, a unique protective wash that mimics intracellular space and provides metabolic support across your workflow, and PluriFreeze PF10, a low-viscosity freezing medium with 10% DMSO that simplifies scale-up and process automation. While we recommend using both products together, PluriFreeze Base can also be used independently during cold-hold steps in your workflow.

Benefits of adding the PluriFreeze Base into your cryopreservation workflow:

- Acts as a hyperosmotic holding solution for cell products: This is useful for large-scale manufacturing processes where processing time exceeds the recommended DMSO exposure time or when maintaining cell products without freezing.
- Easily dilute PluriFreeze PF10 cryomedia to achieve a specific DMSO concentration: Simply mix PluriFreeze PF10 with PluriFreeze Base at the appropriate ratio to obtain the desired final DMSO concentration.
- Use as a wash medium prior to formulating cells in PluriFreeze PF10 freezing medium: With a complementary formulation to PluriFreeze PF10, PluriFreeze Base acts as a protective wash by mimicking intracellular space and providing additional metabolic support.

Note: PluriFreeze Base is not intended to be used as a cell culture medium and should not replace growth medium for quenching steps during dissociation or for maintenance of cells.

Products Used in This Protocol		
	PluriFreeze PF10 (Cryopreservation Media)	**Catalog #:** 22243 (RUO), 23015 (GMP)
	PluriFreeze Base (Hold or Dilution Media)	**Catalog #:** 22244 (RUO), 23014 (GMP)

Storage

- PluriFreeze products are shipped at ambient temperature. Upon receipt, store at 2-8°C, protected from light, until ready to use.
- The solutions inside product bottles and bags are provided sterile and should be handled using proper aseptic technique. If the seal is broken or compromised at the time of receipt, set aside the product and contact us.

Protocol #1: Closed-System PluriFreeze Cryopreservation Workflow

The low viscosity of the PluriFreeze system enables efficient fluid handling and improved washout of residual media components during closed-system, large-scale processing.

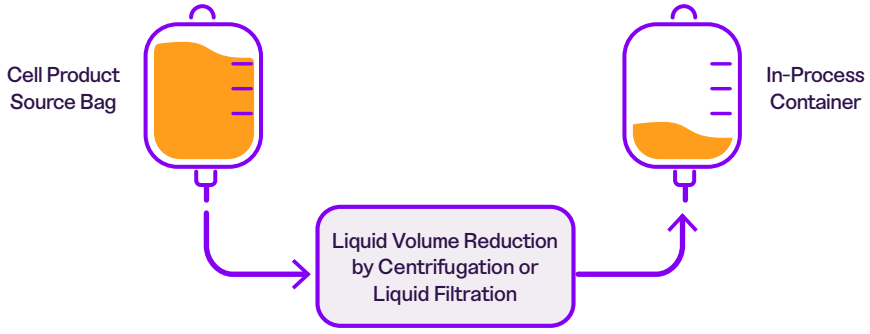
Step 1: Volume Reduction of Culture Media

1.1 To concentrate cells and remove excess growth media, perform a volume reduction using one of the following:

- In-line Centrifugation System (e.g., KSep, Elutra): Spin down and remove supernatant while maintaining a closed loop.
- Liquid Filtration System (e.g., Lovo): Recirculate cells and remove growth media.
- Alternate Method: Use hollow-fiber filters or sedimentation-based devices, if compatible with your cell type.

Ensure most of the original culture media is removed.

Note: Depending on the system utilized, cells may be transferred to an in-process container at the end of Step 1.1 for subsequent washing.

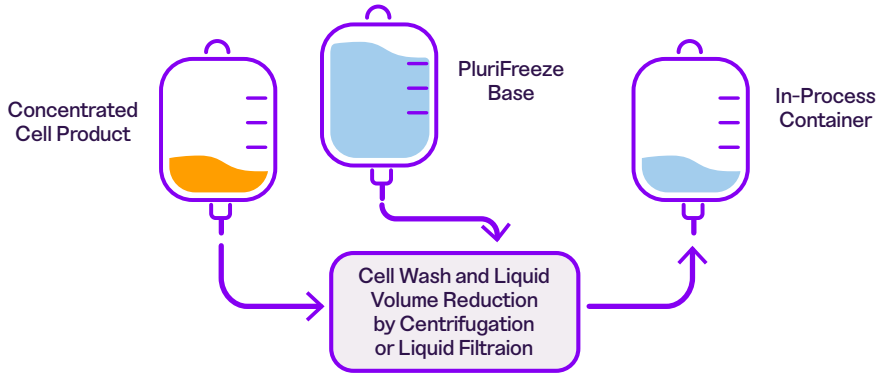


Step 2: Cell Wash with PluriFreeze Base

2.1 Connect the PluriFreeze Base container to the liquid handling system via closed sterile tubing and suspend concentrated cells from Step 1.1 in PluriFreeze Base.

2.2 Perform volume reduction to concentrate cells and remove residual medium.

2.3 Optional: Repeat cell suspension (2.1) and volume reduction (2.2) as needed for additional wash cycles to ensure elimination of residual media.

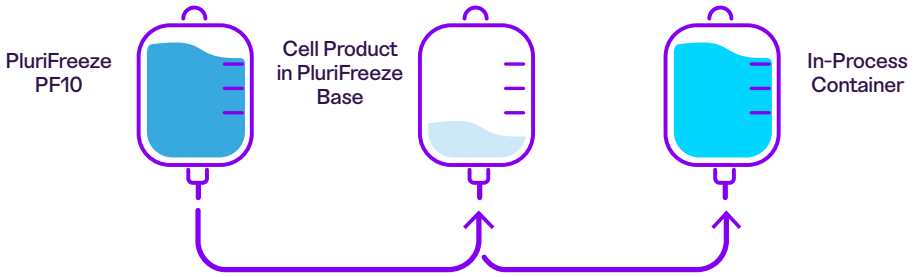


Step 3: Addition of PluriFreeze PF10 Cryoprotectant

3.1 Connect the PluriFreeze PF10 container to the in-process container using a sterile, closed tubing set.

3.2 Transfer the appropriate volume of PF10 to achieve the final desired cell and DMSO concentrations.

3.3 Gently mix the in-process container to homogenize the contents and ensure uniform cell and cryoprotectant distribution.

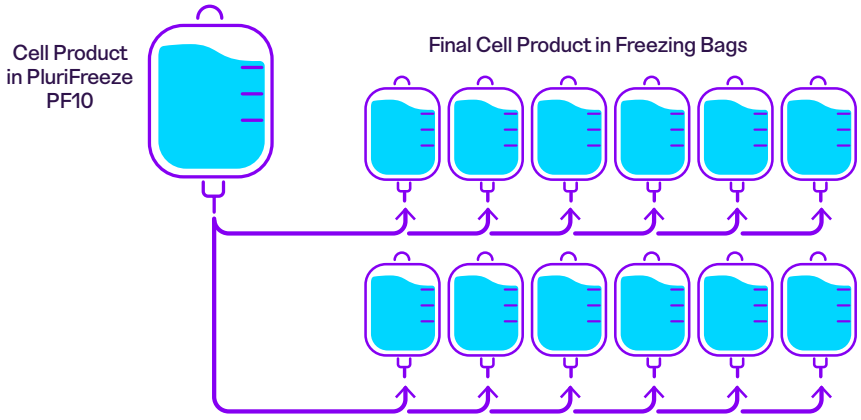


Step 4: Fill, Seal, and Cryopreserve

4.1 Fill freezing bags with the cell product/PF10 mixture, inspect each bag for leaks, and verify that labeling is complete and accurate.

4.2 Seal the freezing bag and remove excess sealed tubing to prepare for freezing.

4.3 Transfer bag(s) to a cryopreservation process (e.g., controlled-rate freezer, vapor-phase nitrogen storage).



Notes and Considerations:

- Perform all steps under aseptic conditions within a biosafety cabinet or using sterile welding/sealing tools to maintain closed-system integrity.
- Volumes, flow rates, hold times, and washout of residual components should be validated for each specific cell type and culture scale.
- Monitor cell viability before and after wash, formulation, and cryopreservation to ensure process effectiveness.

If you require a PluriFreeze formulation with a higher DMSO concentration (up to 30%), please contact us at info@teknova.com.

Protocol #2: Small-Scale Cryopreservation Applications

In small batch processing, PluriFreeze Base can help minimize DMSO and centrifugation stress while allowing for accurate cell counting prior to cryopreservation in PluriFreeze PF10. **Figure 2** shows the process workflow for small-scale cryopreservation applications.

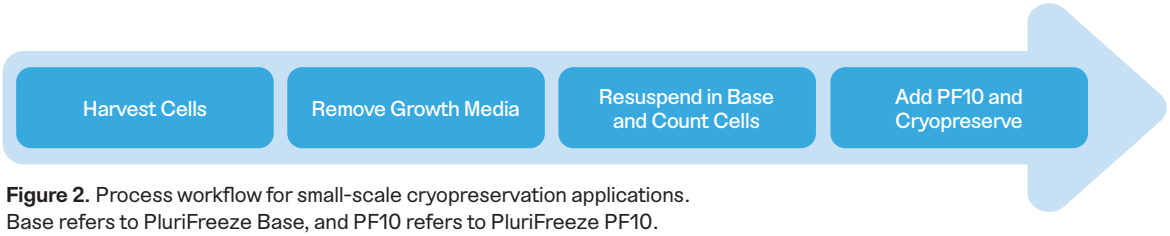


Figure 2. Process workflow for small-scale cryopreservation applications. Base refers to PluriFreeze Base, and PF10 refers to PluriFreeze PF10.

Prior to beginning, ensure that all supplies used for cryopreservation are chilled to 2-8°C.

- If using a controlled-rate freezer, ensure there is a sufficient supply of liquid nitrogen (LN_2) and fill LN_2 vapor-phase carrier to equilibrate to a temperature ≤ 150°C. Follow the manufacturer's instructions for operation of the controlled-rate freezer and carrier. Place a pre-labeled storage box for cryovials into the carrier for temperature equilibration.
- If using an isopropanol freezing container, fill the outer chamber with 100% isopropanol to the fill line and chill at 2-8°C for at least one hour before use.
- Aliquot the appropriate amount of cryomedia (+10% overage) and chill at 2-8°C for at least one hour before cell processing.
- Pre-label and chill cryovials before starting the cryopreservation procedure.

Step 1: Harvest Cells

1.1 Harvest cells using your standard harvest protocol, quenching any dissociation reagent with growth medium.

1.2 Transfer the harvested cells to a conical centrifuge tube.

Step 2: Remove Growth Media

2.1 Concentrate cells: centrifuge cell suspension at a low speed (e.g., 100 x g for 5 minutes at room temperature (15-25°C)).

2.2 Place pre-chilled cryovials on ice or in a thermal rack and transfer to a biosafety cabinet. Gently aspirate the supernatant without disturbing the cell pellet.

Step 3: Resuspend Cells in PluriFreeze Base and Count Cells

3.1 Gently tap the bottom of the tube to loosen the cell pellet.

3.2 Resuspend the cell pellet in a small amount of cold PluriFreeze Base (e.g., 1 mL) and count cells.

3.3 Calculate the required volume of PluriFreeze PF10 to reach the target cell density per cryovial using the formula:

Volume of PF10 = Total Viable Cells / Target Viable Cell Density Per Cryovial (e.g., cell count measured 30 x 10^6 total viable cells, and the target density is 1.5 x 10^6 cells/vial, then 20 mL of PluriFreeze PF10 is needed)

3.4 If necessary, concentrate cells again prior to addition of PluriFreeze PF10 to obtain a final cell suspension volume in 1 mL PluriFreeze Base.

Step 4: Resuspend Cells in PluriFreeze PF10 Freezing Medium

4.1 Slowly add a small volume (~1 mL) of cold PluriFreeze PF10 cryomedia to the sides of the centrifuge tube allowing it to gently flow into the cell suspension.

4.2 Using the same pipette, gently mix by pipetting up and down and swirling the tube until the cell suspension is homogenous. Minimize pipetting whenever possible to reduce shear stress.

4.3 Add the remaining volume of PF10 and mix gently.

4.4 Dispense 1 mL of cell suspension into each labeled pre-chilled cryovial on ice or in a thermal rack.

4.5 Tightly seal the cryovial cap and place the filled vial(s) into a controlled-rate freezer rack or isopropanol freezing container.

Step 5: Cryopreservation of Cells

Controlled-Rate Freezer

5.1.1 If applicable, fill one cryovial with 1 mL PluriFreeze cryomedia to act as an instrument probe.

5.1.2 Following the manufacturer's procedures for operation of controlled-rate freezer, use a gradual cooling rate profile of approximately -1°C per minute, which is often default Program #1 for standard 2 mL cryovials with a 1 mL fill volume.

5.1.3 Once the freezing cycle is complete, quickly transfer the cryovials to a temperature-equilibrated storage box in the LN_2 carrier.

5.1.4 Transfer the frozen cryovials into vapor-phase LN_2 storage.

Passive Freezing Container at -80°C (e.g., Mr. Frosty or CoolCell)

5.2.1 Equilibrate freezing container with cryovials at 2-8°C for 15 minutes.

5.2.2 Transfer the freezing container with cryovials to a -80°C freezer and hold for 15 minutes.

5.2.3 After 15 minutes at -80°C, initiate ice nucleation in the samples by forcefully tapping three times at the bottom and each side quadrant of the freezing container. Return the freezing container to -80°C. Ensure ice nucleation has occurred by visually inspecting ice formation within the cryovials. If ice nucleation did not occur, incubate for additional 5-minute intervals before repeating mechanical agitation.

5.2.4 After 6-24 hours, transfer frozen cells to vapor-phase LN_2 storage.

Protocol #3: PluriFreeze Base Use for Cold Holds and Transport

For cells, tissues, or organ products that don't cryopreserve well and must be transported as live cultures, PluriFreeze Base serves as a hyperosmotic holding solution that can temporarily replace growth medium. Additionally, PluriFreeze Base may be used to hold cells in a quiescent state for in-process analytical testing, particularly in large-scale manufacturing processes. We recommend holding tissues/cells in PluriFreeze Base no more than 72 hours at 2-8°C. Optimal hold time and other parameters should be determined and validated for each particular tissue/cell type.

Note: For all procedures, pre-chill PluriFreeze Base at 2-8°C for at least 1 hour prior to use.

1. Harvest cells or tissues using your appropriate harvest protocol, quenching any dissociation reagent with growth medium.
2. Wash cell product with cold PluriFreeze Base.
3. If necessary, perform multiple wash cycles with PluriFreeze Base to ensure removal of residual growth medium.
4. Resuspend cells or tissues in PluriFreeze Base and proceed with downstream applications (e.g., transporting cells, analytical testing).
5. Store cell/tissue product at 2-8°C. Do not store cells or tissues in PluriFreeze Base at freezing temperatures (< 0°C)—a cryoprotectant such as PluriFreeze PF10 is needed for cryopreservation.
6. If transporting cells or tissues, use a validated shipper to maintain temperature and prevent unwanted temperature excursions.

TIPS FOR SUCCESS

Cryopreservation

- Cryopreservation medium should be chilled to 2-8°C prior to use. Once cells are combined with chilled cryopreservation medium, subsequent processing steps should be performed rapidly to minimize warming of the solution. Exposure time to DMSO prior to freezing should be minimized, ideally under 30-60 minutes, though maximum tolerated exposure time may vary depending upon the cell type and health of cells. Use PluriFreeze Base to provide extended cell processing times prior to addition of the PluriFreeze PF10.
- Cells should be healthy and in log-phase growth prior to cryopreservation. Avoid freezing confluent or stressed cells, or senescent cell cultures. Actively growing cell populations, with high viability, have better post-thaw recovery and viability.
- The optimal cell concentration in cryopreservative depends on cell type but typically ranges from 0.5 to 10 million cells/mL.
- Cells exposed to cryopreservation medium are susceptible to rupture due to osmotic stress. Minimize cell handling stress and avoid centrifugation and vigorous pipetting after combining cells with cryomedium.
- Use of a controlled-rate freezer provides superior consistency, viability, and post-thaw recovery compared to alternative cryopreservation methods. Ensure the controlled-rate freezer program and equipment configuration are appropriate for the specific cryovial or container used.
- To preserve product stability and viability, cryopreserved cellular material must be stored and maintained at temperatures below –135°C (vapor phase LN_2).
- Repeated freeze-thaw cycles of cryopreserved cellular material must be avoided, as they significantly compromise cell viability and product integrity.
- Use LN_2 vapor-phase carriers when transferring cryovials to avoid temperature excursions. Note that dry ice is >100°C warmer than LN_2.

Thawing of Cells

Cells are most vulnerable to degradation from the time the cell vial is removed from LN_2 storage until the cells are placed in growth conditions. Plan and work efficiently to minimize hold times, especially after thaw; use gentle handling techniques and minimize bubble exposure during liquid handling and transfers.

- If resuspending cells in growth medium, warm an aliquot to 37°C prior to thawing cells. Warm an amount sufficient for thaw, as repeated warming or cooling cycles of media may degrade culture performance.
- Use LN_2 vapor-phase carriers when transferring contents to avoid temperature excursions.
- Thaw cells quickly to minimize cell exposure time to DMSO at temperatures above glass transition.
- Use tubing or tips with a larger internal diameter where possible to minimize shear stress on cells. For example, when transferring cell contents out of a 2 mL cryovial, use a 2 mL serological pipette rather than a P1000 micropipettor.
- Dilute cells after thaw to reach a final DMSO concentration of ≤1%, minimizing excessive pipetting or cell agitation wherever possible.

Have a question? Just get in touch.
Our cryopreservation experts are here to help.
info@teknova.com • 1.800.209.4488