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IrvineScientific

# PRIME-XV FreezIS

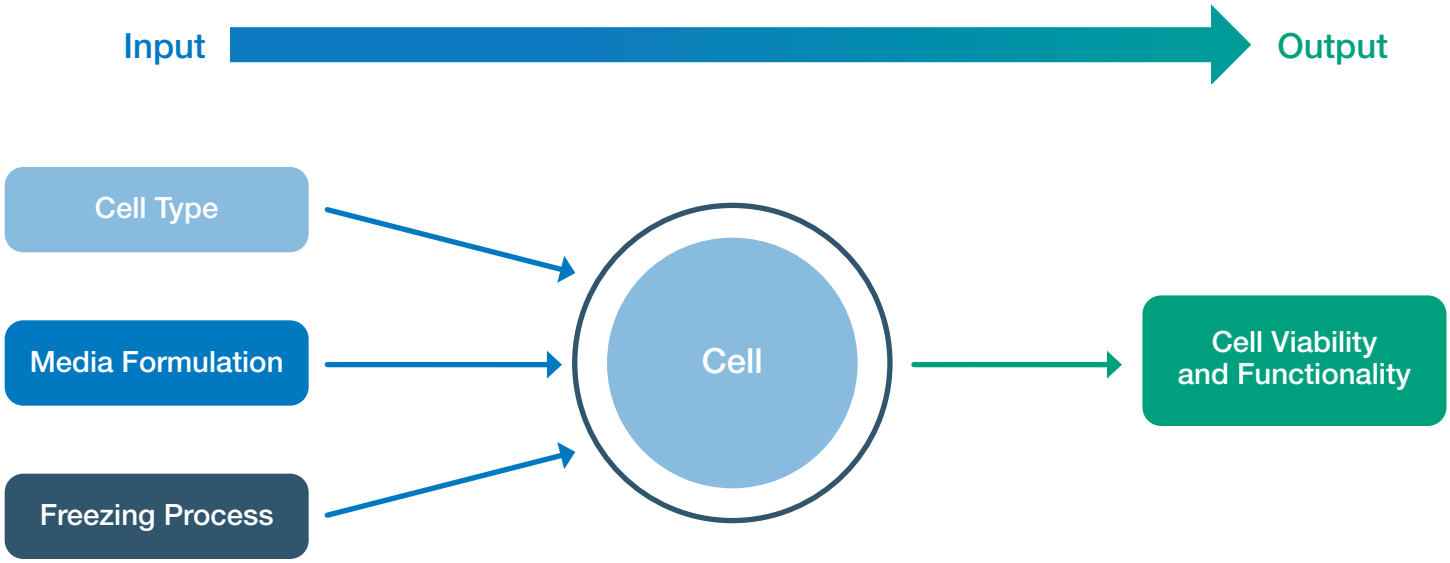
## Cryopreservation Media Solutions for Cell and Gene Therapy Development and Manufacturing

### MINIMIZE RISK WITH GREATER CONTROL

As potential therapies move towards commercialization, the need to produce a sufficient number of cells for effective therapeutic doses, using a well-controlled and optimized process, becomes paramount. In particular, immune cell therapies, using mesenchymal stromal cells (MSCs), hematopoietic stem cells (HSCs), T cells, or natural killer (NK) cells, can require the delivery of 100 million to a few billion cells per dose. As researchers target a wide range of diseases with cell therapy, cryopreservation becomes a critical step that can have drastic impacts on cell viability and potency. Therefore, a careful consideration of the cryopreservation solution will have a significant impact on the final product. PRIME-XV FreezIS cryopreservation media from FUJIFILM Irvine Scientific follows cGMP-compliant manufacturing to ensure a smooth transition to large-scale production, and their chemically defined formulations allow optimal storage and transportation conditions for primary cells.

### KEY FACTORS IN CELL CRYOPRESERVATION

Cell cryopreservation is a process used to preserve cells at ultra-low temperatures, typically between -80 °C and -190 °C. The main purpose of cell cryopreservation is to maintain cell viability and functionality for future use. However, the cryopreservation process can be very harsh and damaging to cells, affecting their ability to proliferate, differentiate, and produce proteins. In particular, cell damage can occur during the freeze-thaw process from oxidative stress, formation of ice crystals, altered cellular structures, osmotic stress, and apoptosis. The success of cell cryopreservation depends on several factors, including: cell type, media formulation, and freezing process.




Sum of the Input = Level of Performance

## CELL TYPE

Understanding that each cell type has different requirements for cryopreservation is crucial. Some cells are more sensitive to freezing and thawing, while others are more resilient. For example, cells with high lipid content, such as adipocytes, are more susceptible to damage during the freezing process. On the other hand, cells with low water content, such as spermatozoa, are more resistant to cryodamage. Therefore, it is important to understand the unique characteristics of the cells being preserved and tailor the cryopreservation protocol accordingly.

## MEDIA FORMULATION

One way to alleviate cell stress during cryopreservation is through the addition of cryoprotectant agents (CPAs) to the media, and there are two types, penetrating and non-penetrating. Penetrating CPAs, such as dimethyl sulfoxide (DMSO), enter the cell through the lipid bilayer to take effect. DMSO forms hydrogen bonds with water and can take the place of water to reduce the overall water content within the cell. This lowers the freezing point of the cells to help avoid internal ice crystal formation. On the other hand, non-penetrating CPAs are often low molecular weight sugars, which will stabilize the cell membrane to reduce the harm of the dehydration process. Both types of CPAs are commonly used in cryopreservation to protect cells, tissues, and organs from damage during freezing and thawing.

Another major part of the formulation is the buffered media which protects against changes in pH caused by the environment, time, and exposure to atmospheric air. The optimal buffer depends on the environmental conditions that the cells are stored in. Components to protect against osmotic stress are also included in the media formulation. Some important factors to consider when selecting a buffered medium for cryopreservation are:

**pH stability:** The pH of the buffered medium should be stable during the freezing and thawing to prevent cell damage and support a wide range of temperatures. Since the media formulation contains buffering systems, it can resist changes to the pH.

**Osmolality:** The osmolality of the medium should be adjusted to meet the optimal range for the cells being preserved. The osmolality affects the rate of water movement in and out of the cells, which can impact cell viability. It can be adjusted using sucrose, mannitol, or sorbitol, but often adjustment of osmolality is not required.

**Toxicity:** It is important to select a buffered medium that is not toxic to the cells being preserved. This can be determined by performing viability assays on the cells after exposure to the medium.

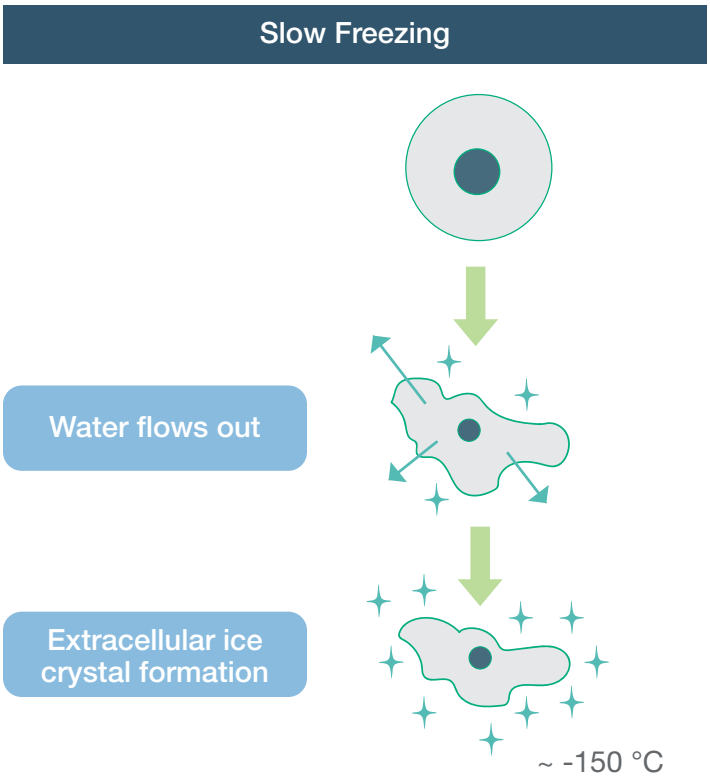
**Cryoprotectant compatibility:** The buffered medium should be compatible with the CPAs being used. Some CPAs are not soluble in certain buffers, while others can interact with the buffer components and cause cell toxicity.

## FREEZING PROCESS

Most cell therapies utilize a slow freezing process. Freezing cells slowly is essential to prevent intracellular ice formation. Unlike embryonic or germ cell therapies, which commonly undergo flash freezing to avoid ice crystal formation, somatic cell therapies require a much larger volume of cells. With larger volumes of cells, the rate of heat transfer becomes a challenge, and requires slow, controlled rates of cooling (~1 °C/min). Additionally, low levels of CPAs are used to minimize toxicity.

As illustrated in the figure to the right, cells dehydrate during a slow freezing process. As water flows out of cells, ice crystals can form on the outside of the cell. However, ice crystals can occur on the inside of the cell as well, which is far more detrimental to cell viability as they can puncture the cell membrane. In addition to ice crystal formation, dehydration of the cells can occur.

Therefore, selecting an appropriate CPA and buffered media is crucial to mitigate osmotic stress; more importantly, it is key to achieving the desired therapeutic output.



## DMSO IN CRYOPRESERVATION MEDIA

One of the most common CPAs in cell therapy manufacturing to protect cells from damage during the freezing and thawing process is DMSO. While it has been shown to be effective, it can negatively affect cells post-thaw and ultimately can impact cell therapy manufacturing downstream. More specifically, DMSO-containing media has been shown to have cytotoxicity and epigenetic modifications in certain cell types, and it can impact cell viability, function, and the consistency of the cell phenotype post-thaw. Using a DMSO-free media can help reduce cytotoxicity, increase consistency, and improve the overall health of the cells.

## CLINICAL STUDIES

Recently, a nonclinical toxicology study was performed using animal models. The aim of the study was to evaluate the toxicity of PRIME-XV FreezIS DMSO-Free, and the proprietary CPA agent that it contains, as a nontoxic replacement for DMSO in cryopreservation media. To effectively investigate the effect of PRIME-XV FreezIS DMSO-Free media, two independent testing laboratories were employed to administer it via various routes in guinea pigs and mouse animal models. Interestingly, the

PRIME-XV FreezIS DMSO-Free media did not show any toxic effects after injection through different entry routes. No clear pathological changes in blood biochemistry or organ histopathology were observed throughout the study in comparison to the PBS control. The animals did not show any clinical signs indicating toxicity, with growth curves and organ weights as expected.

The results of these studies also corroborate the biological and clinical evaluations of the media products used with the Assisted Reproductive Technology (ART) medical device from FUJIFILM Irvine Scientific, which contain the same raw material as the proprietary CPA. The ART media have contact with gametes and embryos during in vitro fertilization and culture, as well as incidental contact with the uterus. These media have been approved by regulatory agencies for over a decade, including the FDA in the US and the EMA in the EU.

To receive and maintain regulatory approval, substantial safety data is required including biocompatibility studies for cytotoxicity, irritation, and sensitization. Furthermore, post-market clinical surveillance data is collected to confirm that there are no long-term safety concerns. Therefore, the results of this toxicology study and the data from the ART medical device should provide reassurance to cell and gene therapy customers when using PRIME-XV FreezIS DMSO-Free media for their cell and gene therapy applications*.[1]

## SUMMARY

To ensure the quality, yield, and function of the final cell therapy product, the freezing process that is developed should utilize high-quality raw materials and follow rigorous quality and regulatory practices. FUJIFILM Irvine Scientific offers a single point of access for critical media, reagent products, and product support that are essential for scaling, such as:

- PRIME-XV FreezIS DMSO-Free media to maintain the potency of Human MSCs, T cells, and HSCs throughout cryopreservation
- PRIME-XV FreezIS DMSO-Free media to maintain cell viability and growth post-thaw with a chemically defined, protein-free formula
- Chemically defined media for enhanced control over media and immune cell manufacturing


References

1. PRIME-XV FreezIS DMSO-Free Cryopreservation Toxicological Non-Clinical Studies in Animal Models Application Note, PN 014359 Rev.03

*PRIME-XV FreezIS DMSO-Free is indicated for research or further manufacturing use

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