NEWS on diagnostics

2025 Volume 1

In this issue....

Welcome to the inaugural edition of News on Diagnostics for 2025! This volume highlights Cryopreservation, a technique for preserving cell lines by freezing them at extremely low temperatures, usually between -80 °C and -196 °C. This method halts cellular metabolism, enabling long-term storage without compromising cell viability. It's especially crucial for cells with limited lifespans.

General Steps in Cryopreservation

- **Preparation:** Ensure cells are healthy and in the logarithmic growth phase. Characterise and check for contamination.
- **Freezing Medium:** Prepare a freezing medium, which usually contains a cryoprotectant like DMSO (dimethyl sulfoxide) or glycerol, a base medium, and sometimes a protein source to protect cells during freezing.
- **Cell Suspension:** For suspension cultures, centrifuge the cells and resuspend them in the freezing medium. For adherent cultures, detach cells gently, centrifuge, and resuspend in the freezing medium.
- **Aliquoting:** Distribute the cell suspension into cryogenic vials.
- **Controlled Freezing:** Freeze the cells slowly at a rate of about -1 °C to -3 °C per minute. This can be done using a programmable cooler or by placing vials in an insulated container in a -70 °C to -90 °C freezer before transferring to liquid nitrogen storage.
- **Storage:** Store the vials in liquid nitrogen (-196 °C) for long-term preservation.

When you need to use the cells, they can be quickly thawed in a 37 °C water bath and then transferred to a suitable growth medium.

Cryopreservation of Cell Lines:

Cell Freezing Tips and Tricks

Ensuring the preservation of cell lines through cryopreservation is crucial for maintaining valuable laboratory resources. Improper freezing techniques can damage cells, disrupt experiments, and negatively impact data collection.

Convenient cell freezing media reagents eliminate the need to titrate DMSO concentrations and are also available in DMSO-free formulations.

CryoStor® cell cryopreservation media is available in 2%, 5%, and 10% concentrations, as well as a DMSO-free formulation, **CryoSOFree™**.

pZerve™ is a cryopreservation solution for serum-containing or serum-free culture that does not contain dimethyl sulfoxide (DMSO), fetal bovine serum or other animal-derived ingredients.

EmbryoMax® 2X Freezing Medium for ES (embryonic stem) cells, formulated with 20% DMSO & fetal bovine serum.

HypoThermosol® FRS Preservation Solution enhances and extends storage of cells, tissues, and organs at 2 – 8 °C.

You can find additional information in our **Online Tutorial.**

CryoProtectants

The best cryoprotectants are those that effectively protect cells from damage during the freezing and thawing processes. Some of the most commonly used cryoprotectants include:

Cryoprotectant	Advantages	Disadvantages
Dimethyl Sulfoxide (DMSO) e.g., D2650	Widely used Penetrates cells and prevents ice crystal formation.	Toxic at high concentrations May cause changes in cellular processes
Glycerol e.g., **G2025**	Commonly used for bacterial and sperm cell preservation. It is less toxic than DMSO	May not penetrate cells as effectively as other protectants
Ethylene Glycol	Often used for cryopreserving embryos and oocytes. Lower toxicity compared to DMSO and glycerol	During the thawing process it can cause toxicity to cells.
Propylene Glycol	Similar to ethylene glycol, used for preserving embryos and other sensitive cells	Can cause osmotic stress to cells, leading to cell damage or death if not carefully controlled
Polyethylene Glycol (PEG)	Helps reduce ice crystal formation often used in combination with other cryoprotectants	High molecular weight PEGs (10 K and 20 KDa) are generally ineffective

Each cryoprotectant has its advantages and limitations, and the choice often depends on the specific cell type and the intended application.

Minimisation of Toxicity

Minimising toxicity during cryopreservation is crucial for maintaining cell viability and function. Strategies to help reduce toxicity include -

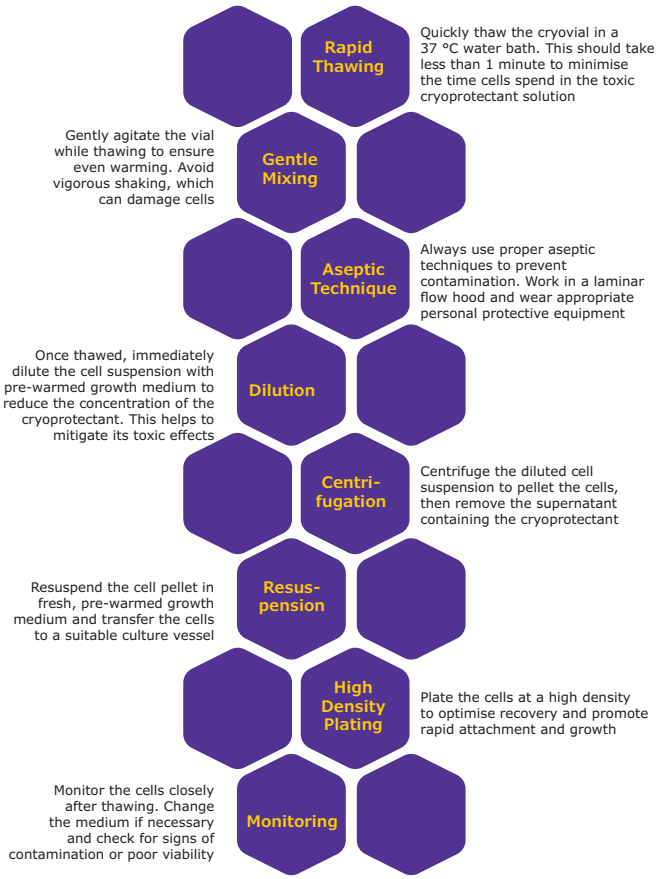
1. **Optimisation of Cryoprotectant Concentration:** Use the lowest effective concentration of cryoprotectants like DMSO or glycerol. Typically, 5-10% DMSO is used, but this can vary depending on the cell type.
2. **Rapid Addition and Removal:** Add and remove cryoprotectants quickly to minimise exposure time. This can help reduce the toxic effects on cells.
3. **Use Combination Cryoprotectants:** Sometimes, using a combination of cryoprotectants can reduce the overall toxicity. For example, combining DMSO with other agents like polyethylene glycol (PEG) or sugars can be beneficial.
4. **Controlled Cooling Rates:** Freeze cells slowly at a rate of about -1 °C to -3 °C per minute. This helps prevent the formation of ice crystals, which can damage cells.

5. **Proper Thawing Techniques:** Thaw cells quickly in a 37 °C water bath to minimise the time they spend in the toxic cryoprotectant solution.
6. **Use Serum or Serum-Free Alternatives:** Adding foetal bovine serum (FBS) or serum-free alternatives to the freezing medium can help protect cells during the freezing process.
7. **Pre-Cooling:** Gradually cool cells to 4 °C before adding cryoprotectants. This can help reduce the shock and toxicity associated with sudden temperature changes.

By carefully managing these factors, you can significantly reduce the toxicity associated with cryopreservation and improve cell recovery and viability.

Thawing Your Cells

Thawing cryopreserved cells properly is crucial for maintaining their viability and function. Here are some of the best practices for thawing cells:



By following these steps, you can maximise the recovery and viability of your cryopreserved cells.

Eppendorf® SmartBlock cryo thaw ideal for thawing samples. Use with Eppendorf® ThermoMixer C & Eppendorf® ThermoStat C

Product Sustainability:
4 Categories of Greener Alternative Products

To determine which products qualify as Greener Alternatives, we quantify sustainability improvements across key impact areas. Each Greener Alternative Product falls under one of these four categories:

Design for Sustainability (DfS) Developed Products demonstrate significant sustainability improvements during our product development process.

Re-engineered Products are improved by our scientists using DOZN™, our Green Chemistry Evaluator.

12 Principles Aligned Products align with at least one of the 12 Principles of Green Chemistry.

Enabling Products help make science greener through enabling technologies.

Our **CryoSOfree™ DMSO-free Cryopreservation Medium**, aligns with the Principle of Green Chemistry "Designing Safer Chemicals" with the removal of DMSO in its formulation.

Explore the full list of Greener Alternative Products by clicking **here**

Stay safe!

When handling cryopreservation, it's crucial to use the appropriate personal protective equipment (PPE) to ensure safety. Here are the key items you should consider:

Cryo-Gloves®: These gloves are designed to protect your hands from extreme cold. They should be loose-fitting so they can be quickly removed in case of an emergency.

Face Shields and Safety Glasses: A full face shield over safety glasses provides protection against splashes and potential exposure to cryogenic liquids.

Long-Sleeved Lab Coats or Shirts: These provide an additional layer of protection for your arms and torso.

Cryo-apron®: Cryogenic aprons made from durable, insulating materials offer extra protection against spills.

Safety Boots: These should be insulated and designed to protect your feet from extreme cold and potential spills.

Labelling your samples

Cryo-labels and markers

Labelling cryopreserved samples accurately is crucial for maintaining sample integrity and traceability.

Use your label for clear and concise information, including essentials such as:

- Sample ID
- Date of cryopreservation
- Type of cells or tissue
- Cryoprotectant used
- Operator initial

We offer a full range of durable labels and markers from Diversified Biotech including **Laser Cryo-Tags®**, **Cryo-Babies®**, **Tough-Tags™**, **Tough-Spots®**, and others. These labels provide coloured, chemically inert, temperature resistant tagging to most plastics, glass, and metals without any degradation. The selection also includes specially designed **Laser Cryo-Tags®** for ease of printing.

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03/2025