

## Product Information

### CryoStor® CS2, CS5, and CS10 Cryopreservation Media

Catalog Numbers **C3124**, **C2999**, and **C2874**  
Storage Temperature 2–8 °C

## TECHNICAL BULLETIN

### Product Description

The CryoStor® CS2, CS5, and CS10 family of preservation solutions represents the next generation of cryopreservation media. Designed to prepare and preserve cells in ultra low temperature environments (–80 to –196 °C), CryoStor media provide a safe, protective environment for cells and tissues during the freezing, storage, and thawing process. Through modulating the cellular biochemical response to the cryopreservation process, these media provide enhanced cell viability and functionality, while eliminating the need to include serum, proteins, or high levels of cytotoxic agents.

CryoStor CS2, CS5, and CS10 are a series of cell-specific, optimized preservation media, uniquely formulated to address the molecular biological aspects of cells during the cryopreservation process; thereby, directly reducing the level of Cryopreservation-Induced Delayed-Onset Cell Death and improving post-thaw cell viability and function.

These media are recommended for the preservation of stem cells, hepatocytes, tissue samples, and other extremely sensitive cell types.

### Reagents

CryoStor CS2 (Catalog Number C3124), CS5 (Catalog Number C2999), and CS10 (Catalog Number C2874) are uniquely formulated cryopreservation media containing 2%, 5%, and 10% DMSO, respectively.

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

The CryoStor media are ready-to-use and complete with no additives required.

Wipe down the outside of container with a 70% ethanol solution before opening as the contents are sterile. If the tamper evident seal has been broken, do not use.

### Storage/Stability

Store the CryoStor media at 2–8 °C and protected from light until ready to use.

### Procedure

#### Cryopreservation with CryoStor Media

1. Suspend cells to be cryopreserved using mechanical or enzymatic dissociation.
2. Centrifuge cells to obtain cell pellet.
3. Remove supernatant.  
Note: Remove as much culture medium as possible to reduce dilution of the CryoStor medium.
4. Isolation - Add **cold (2–8 °C) CryoStor medium** to a cell concentration range of  $0.5\text{--}10 \times 10^6$  cells/ml for standard cell culture protocols. A higher cell concentration is possible with testing.  
Note: CryoStor media contain DMSO, no additives are necessary.
5. Pre-freeze - Incubate cell suspension at 2–8 °C for ~10 minutes.

6. Nucleation – Lower sample temperature to  $-80\text{ }^{\circ}\text{C}$ .
  - a. Use a controlled rate freezer ( $-1\text{ }^{\circ}\text{C}/\text{minute}$ ) or similar procedure for most mammalian cell systems.
  - b. The freezer should be pre-cooled to  $2-8\text{ }^{\circ}\text{C}$ .
  - c. Ice nucleation within the sample (seeding) should be initiated at approximately  $-5\text{ }^{\circ}\text{C}$  using a liquid nitrogen burst program setting on the controlled rate freezer or mechanical agitation (flick or tap) of the cryovial/sample container after 15–20 minutes at  $-80\text{ }^{\circ}\text{C}$ .

Alternative Nucleation Procedures – cells can be frozen using stepwise freezing procedures.

Stepwise freezing procedures include:

- a. 2 hours at  $-20\text{ }^{\circ}\text{C}$  followed by 2 hours at  $-80\text{ }^{\circ}\text{C}$ .  
or
- b. 3–4 hours at  $-80\text{ }^{\circ}\text{C}$  in an isopropanol freezing container. The isopropanol container should be pre-cooled to  $2-8\text{ }^{\circ}\text{C}$

Ice nucleation - mechanical agitation (flick or tap) of the cryovial/sample container after 15–20 minutes at  $-80\text{ }^{\circ}\text{C}$ .

7. Storage – Store the samples at liquid nitrogen temperatures (below  $-130\text{ }^{\circ}\text{C}$ ).  
Note: Sample storage at  $-80\text{ }^{\circ}\text{C}$  is only recommended for short-term storage (weeks to months).

8. Thawing - Thaw samples quickly in a  $37\text{ }^{\circ}\text{C}$  water bath. Sample should be thawed with gentle swirling of the sample until all visible ice has melted. Thaw time for a 1 ml sample in a cryovial is ~3 minutes. Note: DO NOT allow sample to warm above chilled temperatures ( $0-10\text{ }^{\circ}\text{C}$ ). Cryovials should be cool to the touch when removed from the water bath. Passive thaw is **not** recommended.
9. Dilute cell/CryoStor mixture immediately with appropriate culture medium. This can be performed in a single step. The dilution medium should be between  $20-37\text{ }^{\circ}\text{C}$ . A dilution ratio of 1:10 (sample:medium) or greater is recommended.
10. Plate cells appropriately.
11. Culture the cells or use immediately.

## Results

Viability assessment 24-hours post-thaw - Live/Dead fluorescent assays or metabolic assays (MTT or resazurin) are recommended for more accurate viability assessment. Visual inspection of adherent cells and cells “floating” in the medium is also recommended.

Notes: Sample assessment immediately post-thaw with membrane integrity indicators, such as trypan blue for comparative analysis of sample cell yield and viability often results in significant overestimates of cell survival.

To obtain an accurate measure of cell viability following cryopreservation, assessment should be performed 24 hours post-thaw and compared to non-frozen controls.

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