

CryoStor[®] CS2, CS5 and CS10

FREEZE MEDIA

BEST-IN-CLASS, OPTIMIZED BIOPRESERVATION MEDIA FOR CELLS AND TISSUES

Pre-Formulated

Serum-Free

Protein-Free

**USP/Highest Quality
Components**

cGMP Manufactured

FDA Master File

**Sterility, Endotoxin, and
Cell-Based Release Testing**



CRYOSTOR[®]

CryoStor[®], a series of cell-specific, optimized freeze media, is designed to prepare and preserve cells in ultra low temperature environments (-70°C to -196°C); CryoStor[®], pre-formulated with DMSO, provides a safe, protective environment for cells and tissues during the freezing, storage, and thawing process. Through modulating the molecular-biological response to the cryopreservation process, CryoStor[®] provides for enhanced cell viability and functionality while eliminating the need for serum, proteins or high levels of cytotoxic agents.

ORDERING INFORMATION

| <u>Product Name</u> | <u>Size</u> | <u>Part #</u> |
|----------------------------|--------------|---------------|
| CryoStor [®] CS2 | 100mL bottle | 202102 |
| CryoStor [®] CS5 | 100mL bottle | 205102 |
| CryoStor [®] CS5 | 10mL vial | 205373 |
| CryoStor [®] CS10 | 100mL bottle | 210102 |
| CryoStor [®] CS10 | 10ml vial | 210373 |
| CryoStor [®] CS10 | 16mL vial | 210374 |
| CryoStor [®] CS10 | 1L bag | 210210 |
| CryoStor [®] CS10 | 10mL syringe | 210473 |

CryoStor® Usage and Cryopreservation Protocol

- 1) Place cells to be cryopreserved into suspension (*mechanical or enzymatic dissociation*)
- 2) Centrifuge cells to obtain cell pellet
- 3) Remove supernatant - *Note: Remove as much culture media as possible, to reduce dilution of CryoStor® solution.*
- 4) ISOLATION: Add cold (2-8°C) CryoStor®
 - a. Cell concentrations: 0.5-10 × 10⁶ cells/ml for routine cell culture protocols (higher [cell] possible).
 - b. DMSO is pre-mixed in CryoStor® - no additives are necessary.
- 5) PRE-FREEZE: Incubate cell suspension at 2-8°C for approximately 10 minutes
- 6) NUCLEATION: Freeze samples at -70°C (many protocols utilize -70°C and -80°C interchangeably)
 - a. Use a controlled rate freeze (-1°C/min) or similar protocol for most mammalian cell systems.
 - b. The freezing device or isopropanol container should be pre-cooled to 2-8°C.
 - c. Ice nucleation within the sample (seeding) should be initiated at approximately -5°C using either a liquid nitrogen burst program setting on a controlled rate freezer or mechanical agitation (flick or tap) of the cryovial/sample container after approximately 15-20 min. at -70°C.
 - d. Freeze time (-70°C) using isopropanol containers is recommended to be 3-4 hours.
- 7) STORAGE: Place samples into storage
 - a. Store samples at liquid nitrogen temperatures (below -130°C).
 - b. Sample storage at -80°C is only recommended for short-term storage (weeks to months).
- 8) THAWING: Thaw samples quickly in a 37°C water bath
 - a. Sample thawing should be conducted with gentle swirling of sample until all visible ice has melted. Approximate thaw time for a 1 ml sample in a cryovial is approximately 3 minutes.
 - b. DO NOT allow sample to warm above chilled temperatures (0-10°C). Cryovials should be cool to the touch when removed from bath. Passive thaw is not recommended.
- 9) Dilute cell/CryoStor® mixture immediately with culture media
 - a. Dilution procedure can be preformed in a single step.
 - b. The dilution media should be between 20°C and 37°C.
 - c. A dilution ratio of 1:10 (sample to media) or greater is recommended.
- 10) Plate cells in appropriate configuration
- 11) Place cells into culture conditions or utilize immediately
- 12) Viability assessment 24-hours post-thaw*

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

**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.*

Live/Dead fluorescent assays or metabolic assays (MTT or alamarBlue®) are recommended for more accurate viability assessment. Visual inspection of adherent cells and cells "floating" in the media is also recommended.

MATERIALS ARE MANUFACTURED UNDER cGMP

| TEST | METHOD | LIMITS |
|--------------------------|-------------------------------|---|
| Visual Inspection | Visual Inspection | Clear to slightly yellow solution with no visible particulates |
| pH | SOP 3006 | 7.5-7.7 |
| Metabolic Activity Assay | SOP 5100 | Cell viability following preservation is ≥ 75% of cells preserved in the internal standard at Day 1 recovery following preservation |
| Endotoxin | Kinetic Chromogenic USP <85> | ≤ 1 EU/mL |
| Sterility | Membrane Filtration USP <71 > | Sterile |