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

Product Name	Size	Part #
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

BioLife Solutions®
BIOPRESERVATION TOOLS FOR CELLS. TISSUES. AND ORGANS

PRESERVATION CHAIN® Media Products
Source Material — Cell Products

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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 x 10^6 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 utlilize -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

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