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

CRYOSTOR® QUICK REFERENCE 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.

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.

*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.