Video Article

CryoStor Cryopreservation Protocol - ADVERTISEMENT

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Abstract

Cryopreservation efficacy - which includes post-thaw recovery, viability, and functionality is of importance to both research and clinical applications. The cumulative stresses that result from the cryopreservation process and suboptimal freeze media result in cell death from necrosis and apoptosis.[1-5] Cells and tissues can be prepared and preserved in ultra low temperature environments (-80°C to -196°C) using CryoStor cryopreservation solutions. Since these solutions are formulated to address the molecular biological aspects of cells during the cryopreservation process, they reduce the level of cryopreservation-induced delayed-onset cell death, thereby improving post-thaw cell viability and function. In addition, they need to include serum, proteins, or high levels of cytotoxic agents is eliminated with this protocol. In this video article, we will demonstrate the procedures for freezing, storage, and thawing of cells, as well as a viability assessment of the cells post-thaw.

Video Link

The video component of this article can be found at http://www.jove.com/video/2206/

Protocol

1. Preparing Cells for Cryopreservation

1. To prepare for cryopreservation, place cells into suspension by mechanical or enzymatic dissociation.
2. Next, centrifuge cells to obtain a cell pellet.
3. After centrifugation, remove as much of the culture media supernatant as possible, to reduce dilution of the CryoStor solution, which will be added in the next step.
4. Before opening the bottle of cold CryoStor solution, wipe down the outside of the container with 70% ethanol.
5. ISOLATION: Add cold CryoStor to obtain cell concentrations of 0.5-10 x 10^6 cells/mL.
6. PRE-FREEZE: Incubate the cell suspension at 2-8°C for approximately 10 minutes.

2. Freezing and Storing Cells

1. To freeze most mammalian cell systems, use a standard slow rate controlled cooling protocol with a freezing device or isopropanol container pre-cooled to 2-8°C.
2. NUCLEATION: Freeze samples at -80°C.
3. After approximately 10 min. at -80°C, initiate ice nucleation within the sample (seeding) 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.
4. Freeze the cells for 3-4 hours (for isopropanol container).
5. STORAGE: For long-term storage, place samples at liquid nitrogen temperatures, below -130°C. Sample storage at -80°C is only recommended for short-term storage of weeks to months.

3. Thawing Cells

1. Frozen samples should be thawed quickly in a 37°C water bath. Gently swirl the sample(s) until all visible ice has melted. The approximate thaw time for a 1 mL sample in a cryovial is approximately 3 minutes.
2. DO NOT allow the sample(s) to warm above chilled temperatures (0-10°C). When removed from the water bath, the cryovial(s) should be cool to the touch.
3. Dilute the cell/CryoStor mixture immediately with culture media that is between 20°C and 37°C, using a dilution ratio of 1:10 or greater of sample to media.
4. Plate cells in the appropriate configuration.
5. Place cells into culture conditions or utilize immediately.
6. Twenty four hours post-thaw, assess the cells for viability.

4. Representative results

1. Results of cell viability assessment 24-hours post thaw are seen in Figure 1. Compare results of thawed cells with non-frozen controls.

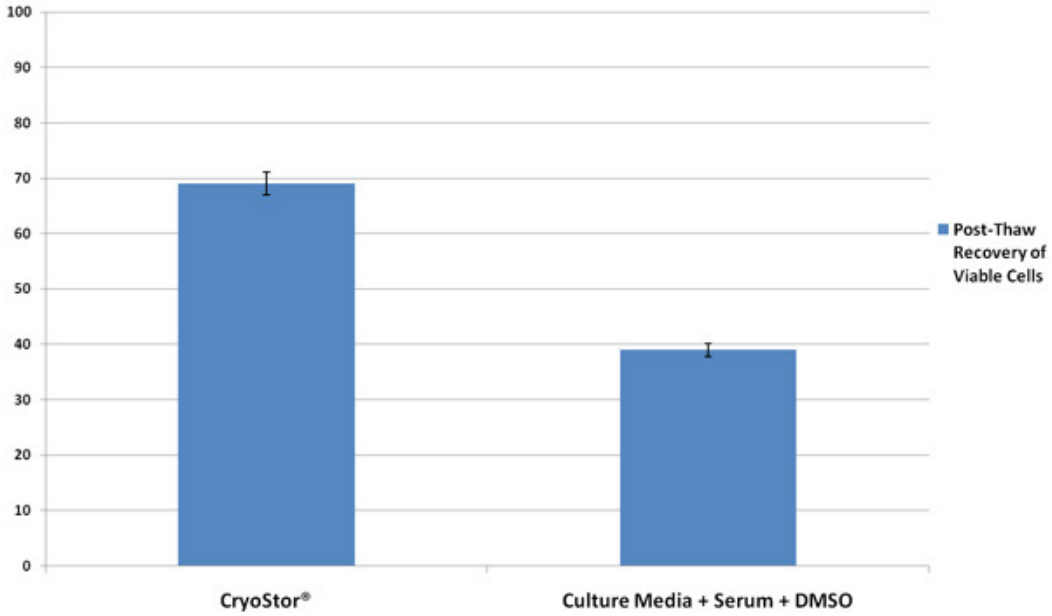

Figure 1. Recovery of human fibroblasts following cryopreservation in traditional culture media/serum/DMSO or the serum-free and protein-free intracellular-like CryoStor: normal human dermal fibroblasts (NHDF) were cryopreserved in culture media/serum/DMSO or in CryoStor cryopreservation media. Following thawing, cells were allowed to recover at standard culture conditions (37°C/5%CO_2/humid air) in fibroblast growth media. Cells were assayed at 24 hours post-thaw with alamarBlue to appropriately assess cell viability following the manifestation of delayed onset cell death processes such as apoptosis and secondary necrosis.

Discussion

This video demonstrated the cryopreservation efficacy of an intracellular-like serum-free and protein-free cryopreservation solution in comparison to a traditional cryococktail made from culture medium, serum, and DMSO. How to cryopreserve cells, the benefits of using an intracellular-like cryopreservation solution, and an example of how to assess the True Viability of cells post-thaw were outlined. It is important to note that because cells die by apoptosis and necrosis post-thaw over a period of hours to days, what is observed as Perceived Viability immediately post-thaw may not be the True Viability long-term. This delayed onset cell death can subsequently impact the quality of cell engraftment and the effective recovery of cellular functional capabilities, both of which are critical to the success of clinical cell and tissue therapies.

Disclosures

Aby Mathew is employed by Biolife Solutions, Inc that produces the reagent used in this article.

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