Efficient Serum-free Cryopreservation of Cell Lines at -20°C and -80°C

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Introduction

Optimizing the cryopreservation of cell lines for short-term and long-term uses promotes biomedical and agricultural research. Current storage techniques involve adding serum in the freezing media and specialized liquid nitrogen facilities, limiting the feasibility of cryopreservation in some facilities. In this study, we used a serum-free commercially available cell cryopreservation medium, C80EZ®, designed for storage in standard laboratory freezers (-20°C to -80°C). C80EZ prevents cryogenic damage through the formation of nano cubic ice crystals (instead of larger, damaging hexagonal ice crystals) during cell storage at -20°C and below. We showed C80EZ's impact on commercially available cell lines: one insect cell line (Sf9) and two mammalian cell lines (MDCK, 293T). C80EZ also protected human coronary artery smooth muscle cells from freezing damage, which is relevant for reconstructive surgeries.

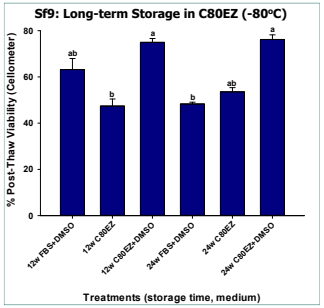
Findings (Insect Cells)

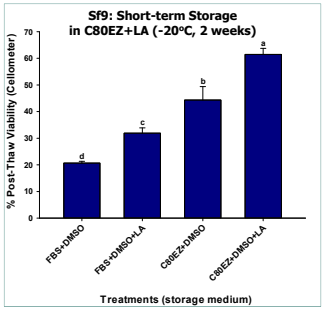
- Flow cytometry & cellometer data showed significantly higher viabilities for Sf9 cells frozen in C80EZ+DMSO (after 24 weeks at -80°C) than all other treatments.
- Combining C80EZ+DMSO with an antioxidant, lactobionate (LA), enhanced cell viability after storage for 2 weeks at -20°C.
- The production of the steroid alkaloid, verazine, in C80EZ cryopreserved cells (1 & 6 months, -80°C) was similar compared to unfrozen cells.

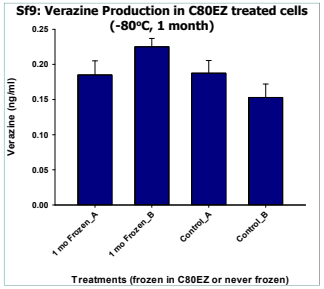
Findings (Mammalian Cells)

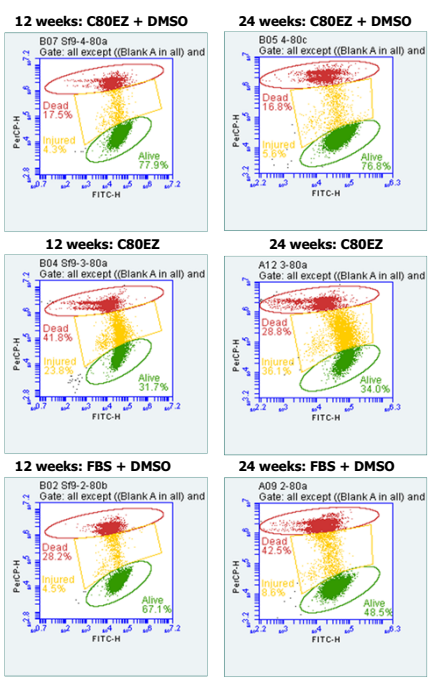
- Human Embryonic Kidney 293 (293T) cells and Madin-Darby Canine Kidney Epithelial (MDCK) cells had 80-90% post-thaw viabilities after storage in C80EZ+DMSO at -80°C for 4-8 months, with control groups having <35% viability.
- Human coronary artery smooth muscle cells frozen in C80EZ for 10 days (-20°C), which were thawed, seeded and grown out for 96 hr, had higher rates of attachment and proliferation than cells frozen in other media.

Insect Cells: Impact of Storage Time and Media on Cell Survival and Verazine Production

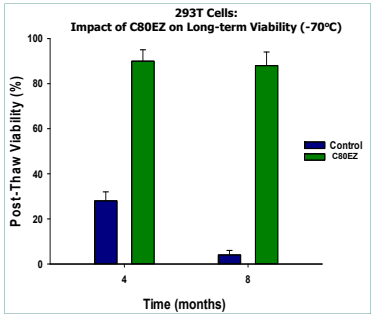


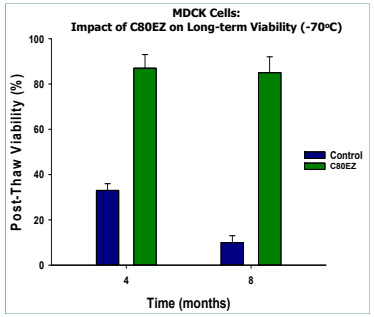




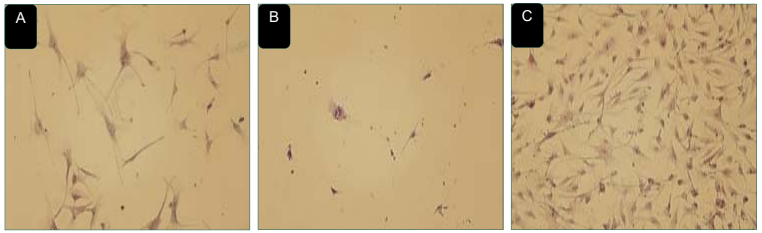


Mammalian Cell Lines: Impact of Storage Time and Media on Cell Survival





Human Vascular Cells: Impact of Media on Cell Growth and morphology



Human coronary artery smooth muscle cells grown out for 96 hours after freezing (-20°C). (A) FBS+DMSO, (B) DMEM+DMSO, (C) C80EZ+DMEM+DMSO. Cells are stained with Diff-quick and imaged at 10x magnification (Khoukaz et al., 2019).

Methods

- Sf9 cells were frozen in C80EZ+serum-free cell culture medium ± 5% DMSO or 90% FBS+5% medium+5% DMSO (control) and stored at either -20°C (directly) or -80°C (using a CoolCell[R]). Addition of the antioxidant, lactobionate (LA), was also tested.
- MDCK and 293T cells were frozen in C80EZ+cell culture medium + 10% DMSO or in MEM with 20% FBS + 10% DMSO (control) and stored at -70°C. HCA-SM cells were frozen in 45% C80EZ + 50% DMEM + 5% DMSO, DMEM + 5% DMSO or 95% FBS + 5% DMSO and stored at -20°C.
- Post-thaw viabilities of insect cells were determined using automated cell counting (Cellometer Vision[R], Nexcelom) and flow cytometery (BD Accuri™ C6); post-thaw viabilities of the mammalian cells were determined using trypan blue, with cell morphology and growth also being monitored.
- For Sf9 cells, the production of verazine (using several recombinant baculovirus constructs) was determined after cells were stored at -80°C.