C80EZ®-TISSUE

Mammalian Tissue Cryopreservation Media (Cat. 502001)

For mammalian cells, insect and competent cells, please use our other products

CryoCrate®
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I. Introduction to C80EZ®

The development of C80EZ® media is based on an invention that applies unique biocompatible polymer combinations to promote nanoscale cubic ice formation. The unique approach minimizes the size of hexagonal ice crystals (the dominating ice structure during freezing of normal aqueous solutions) during freezing and significantly improves the thermal stability of the frozen samples by preventing hexagonal ice regrowth or secondary growth (i.e., recrystallization) during storage and warming. Consequently, all C80EZ® products significantly reduce mechanical damage of tissue structures and fragile cell types, which are majorly associated with ice formation and regrowth during cryopreservation procedures. The anti-apoptosis agents and antioxidants in the C80EZ® media also reduce cell loss due to the use of cell permeating cryoprotectants (e.g., DMSO and glycerol) and excessive intracellular water loss during freezing. Use of C80EZ® hence significantly improves the post-thaw viability and functionality of multiple cell and tissue types that are traditionally difficult to cryopreserve.

With the unique ice structural modification mechanism, application of C80EZ® products also realize long-term storage of tissues in regular laboratory deep freezers (approx. -70 to -80ºC), and do not require liquid nitrogen facilities (approximately -120ºC in the vapor phase and -196ºC in the liquid phase for storage). If the C80EZ® products are used to store tissues in liquid nitrogen facilities, the samples can be safely transported by dry ice boxes (at -78ºC on dry ice surface) instead of using highly expensive and heavy liquid nitrogen dry shippers or dewars. For short-term storage of tissues, C80EZ® products allow users to freeze cells and tissues at -18ºC or -20ºC (i.e., in regular lab freezers) for approximately 10 days without losing viabilities or functionalities, which outperforms most traditional hibernation or hypothermia media. The efficiency of C80EZ®-TISSUE has been tested on mammalian small blood vessels, myocardium tissues, ovarian tissues, etc. All C80EZ® products are serum free and animal protein free. For storage of C80EZ® itself, C80EZ® should be stored at 2 - 8ºC.

II. Cryopreservation Procedures

Due to the fact that it requires a relatively short time for the polymer components in the C80EZ® to permeate through small insect tissues, a pretreatment procedure to achieve thorough permeation is required before DMSO is added.

1. Estimate the volume of the tissue, and prepare at least 10 × tissue volume of the C80EZ®-TISSUE medium.

2. As the pretreatment procedure, merge the tissue into the C80EZ® medium and

    - equilibrate for 30 mins if tissues are thinner than 5 mm
    - equilibrate for 60 mins if tissues are approximately 1 cm or more in thickness;

    this step allows the polymers of C80EZ® to permeate throughout tissues so that the tissue structure can be protected.

3. Mix sufficient (at least 10 × tissue volume) C80EZ®-INSECT with 5% v/v DMSO (i.e. volume ratio of 20:1) to form a complete freezing medium.

4. Load the complete freezing medium into a cryovial (2ml vials for small tissues and 15 ml vials for large tissues), and merge the tissue into the medium in the cryovial. Allow 10 mins of equilibration before freezing.

5. For small cryovials, mount the vials to a freezing kit first, and then cooled in a -80ºC freezer; for large cryovials, directly mount the vials in the -80ºC freezer, for long-term storage.

6. For thawing, the samples are directly merged into an approximately 37ºC warm water bath. DMSO is removed by either direct dilution using 100 × tissue volume of tissue culture medium or holding medium.

For any detailed question regarding the use, please contact us through http://www.cryocrate.com/contact.html by submitting a contact form or call 1-573-884-4576.