

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 cells and tissues in regular laboratory deep freezers (approx. -70 to -80°C), and do not require liquid nitrogen facilities (approximately -120°C in the vapor phase and -196°C in the liquid phase for storage). If the C80EZ[®] products are used to store cells and tissues in liquid nitrogen facilities, the samples can be safely transported by dry ice boxes (at -78°C on dry ice surface) instead of using highly expensive and heavy liquid nitrogen dry shippers or dewars. All C80EZ[®] products are serum free and animal protein free.

C80EZ[®]-2X is specially designed to mix with cell suspensions for cryopreservation, instead of directly adding to cell pellets. It can be used to preserve mammalian cells for both -80°C and liquid nitrogen storage.

For storage of C80EZ[®] itself, C80EZ[®] should be stored at 2 - 8°C.

II. Cryopreservation Procedures

1. A suspension with a high density of cells (e.g., 10⁶ cells/ml, obtained from resuspension of cell pellets obtained by centrifugation, in their culture or holding medium) forms the target suspension, which is aliquoted in cryovials or tubes.
2. C80EZ[®]-2X is mixed with DMSO with a volume ratio of 10:1 to form the complete freezing medium.
3. The new freezing medium is then added dropwise to the cell suspensions in cryovials or tubes (e.g., approximately 0.1 - 0.2 ml per drop for a 1.0 ml sample) by using a pipette in one hand while gently shaking the cryovials or tubes in the other hand, until the final volume ratio between the cell suspension vs. the total freezing medium added is 1:1. The final DMSO concentration is also dropped to 5% v/v, accordingly.
4. For liquid nitrogen storage, the samples are precooled to -80°C by using a freezing kit (e.g., “Mr. Frosty” freezing container www.thermofisher.com/order/catalog/product/5100-0001) in a -80°C freezer overnight, and then transferred into a liquid nitrogen storage tank, or cooled by a programmable cooling machine.
5. For -80°C or -70°C storage, the freezing procedure is as straightforward as using the freezing kit for cooling in a deep freezer for at least two hours, and then later transferring to a sample box in the same freezer.
6. For thawing, the samples are plunged into an approximately 37°C warm water bath. DMSO is removed by either direct dilution using cell culture or holding media, or by resuspension of cell pellets after centrifugation, in compliance with user’s customary protocols.

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.