C80EZ®-NEURON

Mammalian Primary Neuron Cryopreservation Media
(Cat. 503001)

CryoCrate®
life in nano ice

1601 S. Providence Road, Columbia MO 65211 USA
t. 573.884.4576 web: www.CryoCrate.com

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 -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 cells and 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.

C80EZ®-NEURON is specially designed for mammalian primary neurons, for which traditional approaches work poorly. It can be used to preserve neurons for both -80ºC and liquid nitrogen storage.

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

1. Prepare cell pellets by centrifugation of cell suspensions and removal of the supernatant.
2. Mix sufficient C80EZ® with 5% v/v DMSO (i.e. volume ratio of 20:1) to form a complete freezing medium.
3. Directly add the complete freezing medium to the pellets, suspend the cells by pipetting or gentle agitation, to form a new suspension with the cell density on the order of 10^6 cells/ml.
4. The new cell suspension is aliquoted into standard cryovials for cell freezing, typically 1or 2 ml, as complete samples
5. For liquid nitrogen storage, the samples are precooled to -80ºC by using a freezing kit (e.g., “Mr. Frosty” freezing. container www.thermofisher.com/order/catalog/product/5100-0001) in a -80ºC freezer overnight, and then transferred into a liquid nitrogen storage tank, or cooled by any programmable cooling machine.
6. For -80ºC or -70ºC long-term 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 pre-cooled sample box in the same freezer.
7. For thawing, the samples are directly merged into an approximately 37ºC 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.