C80EZ®-INSECT

Insect Cell and Tissue Cryopreservation Media (Cat. 601001)

For mammalian cells and tissues, please use our other products

CryoCrate® life in nano ice
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I. Introduction to C80EZ®-INSECT

C80EZ®-INSECT (Cat.601001) is specially designed for insect cells and tissues. The working mechanism and examples are detailed in www.CryoCrate.com/. The advantages of use include:

- Eliminating the traditional need for high concentration serum (e.g., FBS) for insect cell cryopreservation.
- Enabling long-term storage of insect cells and tissues in the range -70 to -80ºC and it can also be used for liquid nitrogen storage.
- Efficiently reducing insect tissue mechanical damage during freezing and warming procedures, and improving the post-thaw tissue morphology and functionality.

C80EZ®-INSECT prevents insect cell aggregation and maintains most insect cell suspension by itself. C80EZ® requires users to add 5% v/v cell culture grade dimethyl sulfoxide (DMSO) at time of use. The working efficiency has been tested on Sf9, S2, insect ovarian, aorta and other tissue types. All C80EZ® products are sterile filtered (0.22 µm). For long-term storage of C80EZ® itself, C80EZ® should be stored at 2 - 8ºC.

II. Cryopreservation Procedures for Insect Cells

1. Prepare cell pellets by centrifugation of cell suspensions and removal of the supernatant.
2. Mix sufficient C80EZ®-INSECT 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.

III. Cryopreservation Procedures for Insect Tissues

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 is required before DMSO is added

1. Estimate the volume of the tissue, and prepare at least 10 × tissue volume of the C80EZ®-INSECT medium.
2. 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.