Using a CoolCell in a dry ice locker to cryopreserve cells

Introduction

Mammalian cells are cryopreserved to avoid the need for continuous culture, to provide a means of re-establishing an original culture, and to allow easy transfer of a culture to a remote location. Although the cell preservation protocol that provides maximum viability upon recovery may have to be optimized for a given cell line, standardized and repeatable methods will be essential to proper execution of the process. BioCision's CoolCell cell freezing containers provide a convenient and cost effective means of reproducibly conducting the cell freezing process. Although the cell freezing process is typically performed using a mechanical ultra-low freezer, a lack of freezer availability, concerns about frequent freezer access disturbance of the freezing process, or the need for remote or additional freezing capability creates a need for more freezer options. The following procedure describes how to assemble a dry ice locker that will enable uniform freezing of all BioCision CoolCell alcohol-free cell freezing modules.

Materials needed:

- Styrene foam box with lid measuring 18"W x 18"L x 18"H with 2" wall thickness on all sides
- 10" diameter x 10" H ring (such as a metal or cardboard cylinder or aluminum sheeting), or a 10" x 10" x 10" box constructed from cardboard or aluminum sheeting
- Dry ice
- CoolCell cell freezing module

Procedure:

1. Build the dry ice locker
    a. In the styrene foam box, use the 10" ring to create a open space measuring approximately 10 inches around and 10 inches high, or construct a box that is 10 inches in all dimensions with no top side

(see figures 1 and 2 below)

Figure 1: *- External View of Dry Ice Locker*

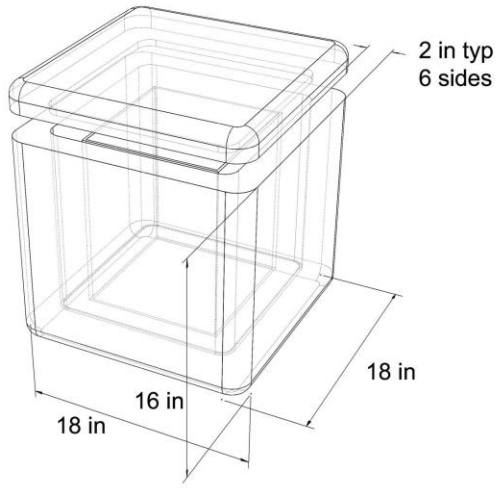
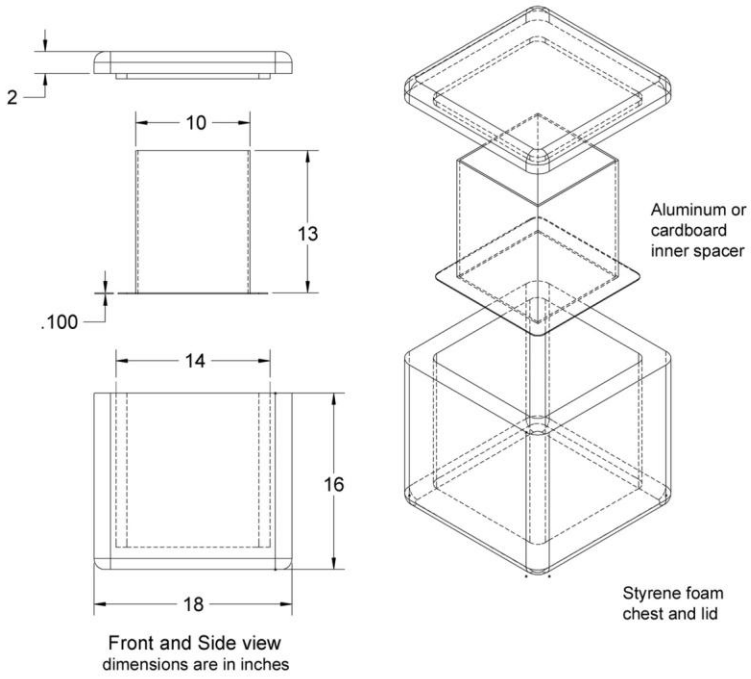


Figure 2:- *Expanded Internal View of Dry Ice Locker*



   b. Completely fill the space outside the ring (the space between the walls of the box and ring) with crushed, pelleted or solid block dry ice.
   c. Place the lid on the box.
   d. Allow 20 minutes for the assembly interior to reach thermal equilibrium (-75^{o}C).
2. Begin cell freezing process
   a. Remove the lid and place a loaded CoolCell into the center of the interior open space.
   b. Close the lid and allow four hours for the CoolCell to reach equilibrium. (The greatest temperature drop occurs in the first two hours. Four hours allows complete equilibrium at the minimum temperature.)
3. Transfer frozen vials to long-term storage
   a. After vials have been in the dry ice locker for four hours, prepare an insulated container (such as an empty CoolBox) with an inch of crushed or pelleted dry ice.
   b. Remove the CoolCell from the dry ice locker and immediately transfer the frozen vials to the dry ice in the insulated pan. (Note: vial contents can rise from -75^{o}C to -50^{o}C in under one minute It is highly recommended that this dry ice transfer step be included to maintain vial temperature and preserve cell viability while transferring to long-term storage.)
   c. Transfer the vials to liquid nitrogen vapor archival storage.
4. Replenish dry ice in the dry ice locker to start another freeze cycle.

Testimonial:-

Sam Knight - Ceramisphere (Australia) *Purely altruistically, I wanted to mention that one big advantage of the CoolCell is that you can use dry ice to do your freezing if you don't have a -80. I spent nearly a year getting terrible viability-recovery on cell freezing with dry ice (in an alcohol device) followed by storage in LN2, and this on HeLa cells which aren't exactly hard to store. I then bought a CoolCell and now I can just use standard cryo-preservation techniques (DMSO, 20% NBS) and get nearly 100% viability on thawing.*

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