Product Applications- Use of Wheaton Cryules for Freezing Cells

Abstract

Cell preservation is an extremely important aspect of cell culture. The only effective means of preserving animal cells is by freezing using either liquid nitrogen or cryogenic freezers. This product application provides a general description for freezing cells and lists materials needed.

Required Materials

Sterile DMSO or Glycerol (see text for sterilization techniques)
Culture Media
Wheaton Glass or Plastic Cryules

Background

Preserving cultured cells differs from preserving bacteria and fungi in that higher viability is required. While a 1% survival rate of a microbial culture is practical, such low viability is unacceptable for cell culture. High survival rates are important for cell lines due to the expense and difficulty in preparation, slow rate of growth, and tendency to change during culture. Consequently, methods used for cell culture cryo-preservation must ensure high viability.

Internal or External Threading- The Great Debate

Internally threaded Cryule vials are perceived to hold a better seal because outside dimensions of the cap and vial are equal. This means that under equal temperature, the cap and vial have expanded the same amount, so the seal should be better. Internally threaded vials also generally have a silicone seal that is also perceived to keep liquid nitrogen out of the vial if it is immersed in the liquid. This makes internally threaded vials popular for long term storage. In practice, the contraction going into liquid nitrogen is so small that it doesn't strain the seal. Although many manufacturers suggest that internally threaded vials can be immersed into liquid nitrogen without harm, many of them sell a sleeve for their vials to prevent liquid nitrogen from entering the seal!
Externally threaded vials, while not so popular for long-term storage, have the advantage that the threads are protected from the environment, reducing the risk of contamination to the culture. The external threading also allows filling to a greater extent than internally threaded vials.

Another Debate- In the Vapor or In the Liquid

Although some cryogenic vial manufacturers say that there is no trouble immersing cultures into liquid nitrogen, most researchers advise against the practice. Although the vast majority of vials seal under liquid nitrogen, there is a chance that the liquid can seep into the vial over time. When the vial is warmed, the trapped liquid nitrogen rapidly becomes a gas, exploding the vial and sending sharp fragments across the lab. In addition to the danger from fragments, the contents of the vial may also be scattered, necessitating decontamination of these areas. In addition, frozen bacteria can also seep in with the nitrogen, contaminating the culture. The main advantage to storing cultures in the liquid is the sense of security that the cells will stay cold in the liquid, even if the freezer is opened. It is advisable to store the cultures in the vapor layer above the liquid. The cells will remain cold provided that proper techniques are applied to opening and closing the freezer. A system should be in place so that cultures can be easily found in the freezer, limiting the time the freezer and cultures are disturbed.

Procedure

Cryo-protectants- choice & sterilization

Cryo-protectants such as DMSO and glycerol are used to prevent cell dehydration while freezing. The cell suspension is generally prepared at a concentration twice that desired for freezing so that an equal volume of cryo-preservative can be added. Gentle handling techniques during harvest and concentration will greatly enhance the viability of recovered cells. Excessive enzyme treatment, vigorous pipetting, and excessive centrifugation will reduce the viability of the cells. Generally, cell viability should be greater than 90% prior to freezing.

The diffusion of cryo-protective agents such as glycerol or dimethylsulfoxide (DMSO) into a cell will partially replace intracellular water and help prevent dehydration due to ice formation while freezing. Glycerol stabilizes proteins in their native states. The cryo- preservative should be prepared separately by combining the cryo-protective agent and the growth medium for the cells. Cryo-protective agents are usually used individually in concentrations ranging from 5-15% (v/v) with the optimum changing with cell type. Use the highest quality cryo-protective reagents, and sterilize them before use. Glycerol can be sterilized by autoclaving for 15 minutes and stored in small aliquots to prevent contaminants into the bulk material. DMSO is sterilized by filtration with a 0.2 um nylon syringe filter then stored at -20°C in small, single-use sealed aliquots. Air oxidation of DMSO is rapid and these oxidation products are toxic to cells. Cells mixed with cryo-preservative require an equilibration time at room temperature prior to freezing. This equilibration period ranges from 15 to 45 minutes and allows the cryo-preservative to penetrate the cell for maximum protection.

Sealing Glass Cryules

Glass Cryules are sealed with a flame by rolling the sealing neck of the Cryule in a flame until it becomes soft and pliable. Use forceps to slowly pull the sealing neck of the Cryule while continuing to roll the tube. As the top of the sealing neck separates from the vial, roll the tip of the Cryule in the flame to seal. After the Cryule has cooled, it can be submersed in a solution of methylene blue or trypan blue in order to check that the Cryule has sealed. Wash the Cryule to remove the stain and examine the cell suspension for the dye. Any dye on the inside of the Cryule means the vial was not sealed and should be discarded.

Initial freezing & storage

The rate of cooling controls the size of the ice crystals and the rate at which they are formed, both of which affect cell recovery. In most cases a slow, uniform cooling rate of -1°C per minute from ambient works well. Placing the vials in a disposable plastic polystyrene rack (page 46,) on the shelf of an -80°C freezer for 2-3 hours is close to 1°C per minute and works well for a range of cell types. After initial cooling, the cells are placed in the storage freezer. For Glass Cryule vials (page 43), use the Disposable Plastic Racks on page 46- tilt the Glass Cryule in the rack so the culture freezes at a slant. This reduces the chance of the expanding ice cracking the Cryule. Whether using Wheaton plastic or glass Cryules, simply leaving the culture in the –80 °C freezer overnight is a good way to initially freeze the cells. The temperature at which frozen cells are stored will affect their viability. Storage at -80°C may permit slow chemical reactions from to small amounts of unfrozen water, resulting in cell death. Cells should be stored at a temperature less than –150 °C

Thawing

In contrast to freezing, rapid thawing of cells is needed to maintain viability. When removing vials from the freezer, insulated gloves should be worn to protect yourself from burns from the low temperatures. Though specially designed Cryules are used to store cells, a face shield and laboratory coat help to protect you against explosions. Directly after removal from storage, vials should be thawed with agitation, except for fragile hybridoma cells, in a 37°C water bath. As the last ice crystals melt, remove the vial from the water. Wipe, spray, or submerse the vial with 70% ethanol before opening it in a biosafety hood. You can determine the percentage of viable cells