Q&A ATCC® *Excellence in Research* Webinar "Best Practices in Cryopreservation"

General Questions

1. Will we be able to download the presentation?
   This presentation is available to watch on-demand on ATCC's website, or click here.

2. Why is it necessary to thaw cells quickly?
   The dimethyl sulfoxide (DMSO) in most cryopreservation reagents is toxic to cells after they have thawed. Quick thawing reduces the exposure of the cells to DMSO. When thawing, the addition of media will dilute the DMSO present; however, it is necessary to add the media slowly to avoid osmotic shock to the cells. Therefore, it becomes necessary to thaw cells quickly to minimize the time of exposure of the cells to DMSO.

3. Do Styrofoam coolers work as well as the CoolCell® LX Alcohol-Free Cryopreservation Container (ATCC® ACS-6000™)?
   We do not recommend using Styrofoam containers to freeze cells because it is difficult to achieve a consistent rate of temperature drop. The CoolCell® LX Alcohol-Free Cryopreservation Container (ATCC® ACS-6000™) is a specially designed insulated freezing container that maintains the optimal controlled cooling rate of -1°C per minute when placed in a -80°C freezer and allows you to transfer the vials from the CoolCell LX chamber into the liquid nitrogen tank after four hours at -80°C. Its polyethylene shell, the metal conductor, as well as physical size and dimensions, make it especially designed to achieve that rate. The best method uses a computer controlled, programmable electronic freezing unit (e.g., CryoMed Freezer; Thermo Fisher Scientific) which rigorously maintains this rate of cooling. This is the method used exclusively at ATCC. Such equipment is expensive and is only absolutely necessary for the most sensitive cells.

4. With the CoolCell, should you precool the container before adding the cells?
   No, this step is not required. The CoolCell shell allows you to add the cryovials of cells to the chamber, at room temperature and then directly transfer the vials into the liquid nitrogen tank after four hours at -80°C. After you remove it from the freezer, you can simply add another batch of cells into the CoolCell chamber, you do not have to wait for it to warm up.

5. We are sure that we are following proper freezing techniques, but are still seeing low viability after thawing. What could account for this?
   There are many critical factors that will affect the growth and viability of cells upon thaw. Some of the most important include the dissociation process prior to freezing, the freezing process itself, composition of the freeze medium, the growth phase of the culture upon harvest (ideally cells must be in exponential growth), the stage of the cell in the cell cycle, and the number and concentration of cells within the freezing solution. A comprehensive cryopreservation manual is available for download on the ATCC website.
   If you followed proper freezing techniques, you should evaluate your cell dissociation process. We have found that certain cell types, such as primary cells, are more sensitive to trypsin. In this case, it is recommended to use solutions with a lower percent of trypsin, such as Trypsin-EDTA for Primary Cells (ATCC® PCS-999-003™) and Trypsin Neutralizing Solution (ATCC® PCS-999-004™) Even more sensitive cells may even need a dissociation agent that contains no enzyme and is free of animal products, such as Non-Enzymatic Cell Dissociation Solution (ATCC® 30-2103™).

6. What kind of viability should we expect for primary and stem cells?
   For ATCC's primary and stem cells, you should expect 70% or greater cell viability, if our recommendations for culturing are closely followed. Some cell lines do not recover well from cryopreservation and may typically only have 30-40% viability after thawing. This isn't considered a major problem since these cell lines can often expand quickly in lower densities if initiated in the correct complete growth medium and a high quality non heat-inactivated serum is used such as Fetal Bovine Serum (FBS) (ATCC® 30-2020™).

7. What about freezing algal cells? Could you describe the media used, including the concentration of the cryopreservant?
   For algae, the preservation method really depends on the organism and the cryprotectant and concentration is specific to the genus. There is no one size fits all cryopreservation method that would work for all algae. While DMSO and Methanol are commonly used, some species can be preserved with 7.5% Glycerol. For DMSO and Methanol, the concentration range is usually between 2.5% and 12.5%. You can download the ATCC® Protistology Culture Guide for more details. Page 22 of the guide has a table with this information.

8. I work in a lab that stores viruses at -70°C and -120°C for long term storage. Would it better to store these viruses at -80°C?
   Free virus storage should be fine at and between -70°C and -120°C. Most people find storing material in a -70°C or -80°C freezer to be more convenient since this temperature is sufficient.

Storing at a colder temperature (-120°C) is certainly fine. Viruses in infected cells can be stored at -140°C with 7% DMSO and 10% FBS.

9. Have you tried different cryoprotectants than DMSO and compared viability data?
   For best results we recommend using standard growth medium with 5-10% Dimethylsulfoxide (DMSO) (ATCC® 4-X™) or Serum-Free Cell Freezing Medium (ATCC® 30-2600™). At ATCC, we successfully use these reagents to cryopreserve a wide array of both adherent and suspension cell lines. They have been fully tested to support cell viability and growth post-thaw.
   ATCC DMSO is cell culture grade and each lot has been tested on ATCC cell lines to ensure it is nontoxic when used at concentrations of 10% or less as well as to assure nontoxicity and sterility. Cells cryopreserved using Serum-Free Freezing Medium show levels of viability and percent attachment (adherent cells) that are comparable to cells preserved in DMSO and FBS. Serum-Free Cell Freezing Medium can be used for both cells cultured in serum-supplemented growth medium as well as cells grown under serum-free conditions.

10. What is better to autoclave or filter DMSO? Why?
    It is generally recommended to filter DMSO rather than autoclave it. We recommend using ATCC DMSO, which is a ready-to-use sterile filtered solution.

11. Is there a standard loss in cell viability even when freezing at the right rate?
    Generally, for stem cells and primary cells purchased from ATCC, you can expect post-thaw cell viability to be 70% or greater if the specific protocol for that particular cell is followed. For some specific cell lines, viabilities could be as low as 30-50%. This is not a problem as most cell lines will recover and expand easily when plated according to the cell specific protocol.

12. What are the effects of cooling at a rate that is slower than one degree a minute?
    At a rate much lower than one degree per minute, ice crystals could form within the cells, causing cellular damage and leading to low viability upon thaw. The aim of cryopreserving cells at an optimal cooling rate is to prevent formation of intracellular ice crystals. When cells are “slow cooled” ice forms in the extracellular space causing concentration of solute in the residual water. This can result in an osmotic imbalance between the inside and outside of the cell. The rate of cooling must be sufficiently slow to keep the osmotic imbalance to a minimum. For more information, see Mazur P, Cryobiology: The Freezing of Living Cells: Mechanisms and Implications. Am J Physiol 247:C125-C142, 1984.

13. Is there a harm of immersing a cryovial of cells in liquid nitrogen?

    There are two basic types of liquid nitrogen storage systems: immersing vials in the liquid or holding vials in the vapor phase above the liquid. The liquid phase system holds more nitrogen and thus requires less maintenance. However, there is always a chance that some liquid will enter improperly sealed vials and may cause the vial to explode when retrieved. Also, as a safety concern, retrieving cells from direct liquid nitrogen could cause harmful skin exposure to the cold liquid if proper PPE is not used. For these reasons, ATCC strongly recommends storage in vapor-phase systems. Vapor phase systems create a vertical temperature gradient within the container. The temperature in the liquid nitrogen at the bottom will be -196°C, whereas the temperature at the top will vary depending upon the amount of liquid nitrogen at the bottom as well as the amount of time the container is opened.

    To ensure safe storage of your cells, be sure to keep enough liquid nitrogen in the container so that the temperature at the top (just above the storage level) is -130°C or lower. All storage systems should be equipped with temperature alarms. Assuming the vial does not get damaged, there should be no harm to the cells at this direct exposure.

14. Do stem cells, neurons, and sperm need special preservation?

    If the standard animal cryopreservation techniques and rapid thawing recommendations are properly followed, these cell types do not require any special preservation compared to other cell types. Please refer to the ATCC Culture Guides for more helpful information.

15. Why should we place the cells at room temperature for 15 minutes?

    This step occurs after the cells have been initially placed in the Freeze Media, before freezing down cells. This step is to allow the cells to equilibrate with the media and allow the DMSO to completely penetrate the cell membranes and permeate the cells. Do not overexpose the cells to DMSO by exceeding the time over 40 minutes. At this point, cells could lose viability due to the toxicity of DMSO.

16. What is the best method to thaw bacterial stock from cryopreserved stock?

    Thawing frozen bacterial stock can be done using the same method as thawing animal cells, using a warm (often 37°C) water bath for approximately 1-2 minutes only. Different bacteria grow at different temperatures so be sure to keep this in mind and not leave the cells at an inappropriate temperature for too long. More detailed information can be found in the ATCC Bacterial Culture Guide, which can be downloaded on our website.

17. Is 90% FBS + 10% DMSO better than medium +20% FBS + 10% DMSO?
    ATCC routinely uses complete culture medium + 5-10% DMSO to freeze cells. The recommended freezing medium for any ATCC cell line can be found on our website in the Cryopreservation section under the Culture Method tab. There is no disadvantage to using a 90% FBS + 10% DMSO freezing solution if you prefer for most cells.

18. We would like to quantify operating costs for long-term storage, do you have any good resources for estimating cost differences between -80°C and -140°C?
    Your operating costs will be dependent on the kind of equipment you will be using for long term storage. Commercial manufacturers of both -80°C and liquid nitrogen freezers will provide detailed electrical specifications on which you should base your estimated costs. If costs of maintaining your own cryostocks are a limitation, ATCC offers both cGMP safe deposit and Standard safe deposit services for secure, confidential large-capacity storage of cell lines, microorganisms, and other biological materials. Under this service, all materials are stored in liquid nitrogen tanks equipped with monitoring systems and 24-hour surveillance to safeguard stored material.

    Please note: animal cells cannot be stored at -80C for longer than 24 hours.

19. Can we preserve patients derived xenografts (PDX) in mouse tumors with this method, any important instructions for this? What is difference between patients derived xenografts and patients derived long term culture cryopreservation?
    Both cryopreservation methods, the “Serum/DMSO” protocol and the “vitrification” protocol can be used; however, it is important to maintain the PDX model at a relatively low passage number to ensure that the genetic and biochemical integrity of the original tumor material is preserved.