Science Education Collection

Regulating Temperature in the Lab: Preserving Samples Using Cold

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Abstract

Preservation of laboratory samples, specimens, and reagents using extreme cold is routinely performed in biomedical research labs. This video will discuss some of the methods for keeping laboratory samples cold and will explain the correct cooling method to use for each experimental requirement.

For example, cooling agents, such as ice and dry ice, are typically used when keeping samples cold during experiments. This video discusses the physical properties of the most commonly used cooling agents, as well as safety precautions for working with them.

When it comes to keeping samples cold in between experiments, cooling equipment, including laboratory grade refrigerators and freezers can be used to preserve samples for extended period of time. Also discussed in this video are types of samples and reagents that can be stored in the commonly-available laboratory cooling equipment.

Finally, the concept of cryopreservation is introduced as a process through which tissues, cells, and biomolecules are cooled to sub-zero temperatures, thereby effectively stopping all sample-degrading biological activity. Several methods of cryopreservation are discussed that minimize or eliminate the formation of damaging ice crystals.

Transcript

Preservation of laboratory samples, specimens and reagents, is a requirement of research laboratories worldwide. An efficient way to preserve sample integrity and viability over time is by maintaining them at cold temperatures.

Whether you are working with a sample at the bench, or storing a sample at the end of an experiment, different methods of cooling can be used. This video will demonstrate the types of cooling agents and instruments typically found in the lab and will help you understand what types of samples are stored at which temperatures.

The choice of cooling agent is dependent on the nature of experimental procedure being performed.

Conventional ice is the logical choice for preserving samples over the short-term. You probably know that ice is frozen water, which has a melting point of 0 °C at normal atmospheric pressure as you can see in this phase diagram. You might not know that it is sometimes referred to as “wet ice”, because it becomes liquid as it warms at room temperature.

“Wet Ice’ is ideal for keeping samples and reagents cold while working with- or transporting them.

While “wet ice’ is solid H2O, “dry ice’ is the solid form of carbon dioxide, which has a melting point of -78.5 ˚C. Dry ice does not melt into liquid at atmospheric pressure but rather transforms directly into carbon dioxide gas, through a process called sublimation. Sublimation refers to a shift in the phase of matter from solid directly to gas and occurs below the triple point in a phase diagram.

Use dry ice, when you are working with biological specimens such as frozen bacterial or mammalian cells or tissue, which are generally stored at temperatures well below 0˚C.

Dry ice is also advantageous as it leaves no residue upon changing state, which makes it ideal for constructing a freezing bath by pouring liquid over dry ice.

Liquid nitrogen is condensed nitrogen gas and is. commonly written as “LN2”. At atmospheric pressure liquid nitrogen boils, or transitions from liquid to a gas, at -196 °C, which you can see by its phase diagram.

When you need to store biological specimens at temperatures below what most laboratory freezers can obtain, liquid nitrogen is used.

Liquid Nitrogen can be stored in a dewar, or vacuum flask, with a loose fitting lid or a large tank dewar equipped with a relief valve to prevent pressure build-up within the system.

Though non-toxic, dry ice and liquid nitrogen are dangerous materials and should not be handled until you have been trained by an experienced member of the lab.

Due to the extremely low temperatures of liquid nitrogen and dry ice, severe tissue damage can occur upon contact with skin. Always wear proper protection, including cryogenic gloves and a lab coat. Use tools to manipulate samples to avoid contact with skin.

Also, airtight containers should never be used to store either dry ice or liquid nitrogen, since these cooling agents change state into gas. Under airtight conditions, pressure can build leading to an explosion.

And now for the instruments that keep samples cold… Laboratory refrigerators and freezers regulate temperature more tightly than those found in the home to ensure a uniform temperature throughout the unit.

They are generally equipped with temperature monitoring systems and alarms that go off following significant temperature change.

Never store food or drink in lab fridges or freezers, as this could result in contamination with toxic chemicals or bacteria. You'll have to find another place to store your lunch.

Refrigerators are maintained at 4°C and generally used for temporary storage of samples especially when freezing can affect sample integrity.

Many reagents and solutions are stored at 4°C to extend their shelf life, including tissue culture media and poured cell culture plates, which are warmed before use.

Cold rooms are ideal for storage of larger equipment that should operate at low temperatures, such as liquid chromatography units.

Laboratory grade freezers range in temperature from -20° C to -196 °C for cryogenic freezers.

For storage of nucleic acids and reagents, such as restriction enzyme, -20 °C is the appropriate choice. Upon removal from the freezer, samples and reagents should be kept on ice.

-80°C and cryogenic freezers are suitable for storage of frozen tissue and cells over an extended period of time following cryopreservation in liquid nitrogen. Dry ice is generally used to transport samples taken out of -80 ˚C freezers.

Cryopreservation is a term that refers to the long-term storage of tissues, or even living cells. At sub-zero temperatures all biological activity, including reactions that degrade the sample is effectively stopped.

When freezing living cells and tissue, ice crystals can form, leading to cell dehydration and damage, as well as accumulation of solute molecules to harmful concentrations.

Snap- or flash-freezing is the process by which biological samples are rapidly submerged in liquid nitrogen, or a mixture of dry ice and ethanol, so that large ice crystals cannot form and damage the cells. Cryoprotectants can also be used as an additive to reduce the formation of ice.

As an alternative to flash freezing, machines can be used to slowly control the freezing process, which is needed to cryopreserve the sheep embryos you see here.

Recently, vitrification has been in introduced as a method to cryopreserve cells and tissue without any damage due to ice crystals. This process transforms the liquid in the sample to a non-crystalline, glassy solid through rapid cooling in the presence of certain cryoprotectants.

You've just watched JoVE's introduction to cooling laboratory specimens and reagents.

In this video we reviewed different types of cooling agents and equipment, and examples of when to use each cooling method. We also introduced several ways to cryopreserve biological specimens. Thanks for watching.