Technical Notes

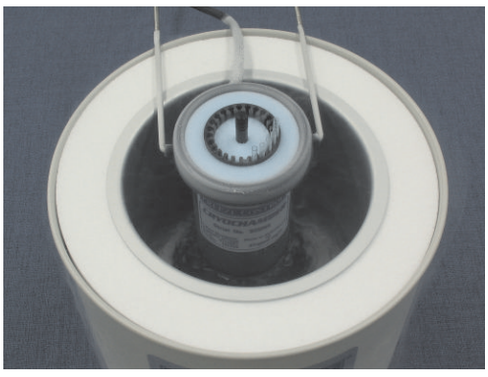
FREEZE CONTROL®

Operating Principles of FREEZE CONTROL® Systems

Key aspects in the operation of FREEZE CONTROL® systems include:

- Cryochambers stand directly in liquid nitrogen. The cryochamber, therefore is at a stable liquid nitrogen temperature of -196°C. Only a small quantity of liquid nitrogen is required to maintain appropriate performance.
- Heating is provided to precisely regulate the temperature of the heat exchange substrate.
- Temperature of specimens is continuously monitored and adjusted in a stable way to precisely match the selected protocol. This is achieved through a highly sensitive feedback loop between the cryochamber and the temperature controller.
- A high degree of temperature stability is maintained, and specimens remain within the controlled temperature of the cryochamber at all times.
- Heat is removed from specimens rapidly and efficiently through tight coupling of specimen containers with the heat exchange substrate.
- Temperature uniformity between and along each specimen column is achieved through the highly conductive material used in the cryochambers, their concentric design, and custom designed heating element.
- The cryochamber is used in a vertical freezing position which can help to predict the position of the specimens.

A good understanding of the way FREEZE CONTROL® systems operate will help in developing procedures which are optimal with these systems.



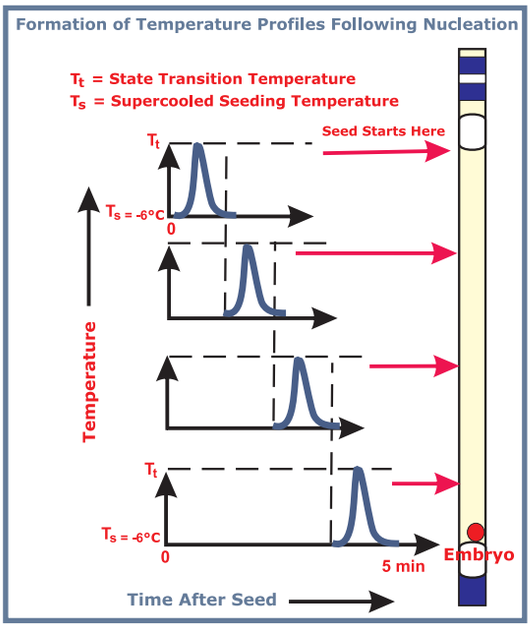
Ice Nucleation in a Vertical Straw

Nucleation is the onset of a change of state from liquid to crystalline. Initiating ice nucleation (seeding) is associated with an energy change. Immediately following ice nucleation the temperature will rise where ice has formed and an ice front can be observed to propagate down the straw, resulting in a wave of so called latent heat peaks (as shown in the diagram). A localised rise in temperature, known as the latent heat of fusion occurs as the system returns to the supercooled seeding temperature.

In a vertically positioned straw, the embryo will slowly sink towards the bottom meniscus of the straw column. This provides a precise and known location of the embryo.

As the ice propagates down the straw to the bottom meniscus, the ice will first pass along the wall and to the meniscus, lifting the embryo towards the centre and upwards, until the rest of the ice forms and surrounds the embryo.

Seeding is always recommended to be carried out away from the embryo to prevent the onset of cellular damage from ice formation within its structure. Seeding is easy and reliable with our vertical system, as the straws are lifted just high enough to expose the top meniscus of the specimen column for seeding, while the part with the embryo remains inside the core of the chamber at constant temperature



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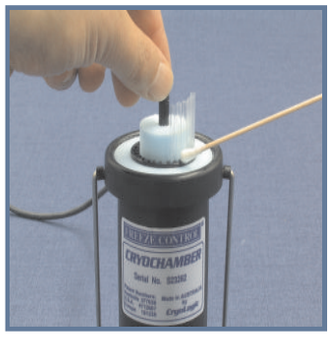
Guide to Manual Seeding

When using FREEZE CONTROL® technology ensure that the seeding temperature is no more than 2°C below the media's freezing point. It is strongly recommended that specimens are held at the seeding temperature for no longer than one minute before manual seeding is performed to reduce the risk of self-seeding.
After initiating seeding, specimens are held at the seeding temperature for the remainder of the hold period before the program progresses. The important role of the hold period is to allow the medium to become frozen (ice nucleation process), and to allow the first stage of dehydration to be completed well before the cooling ramp continues. As the latent heat is released, the design of, and material used in, the cryochamber, ensure that heat is uniformly, efficiently and quickly removed from the specimens.

STEP BY STEP MANUAL SEEDING

- Perform manual seeding when the temperature is at the seeding plateau (commonly between -5°C to -6.5°C) of the freezing program
- Allow approximately 1 minute for specimens to equilibrate to the seeding temperature
- Remove the cryochamber lid
- Raise the specimens with the lifter until the upper meniscus of the column with the specimens is just exposed
- Briefly apply a cotton swab, spatula or forceps dipped in liquid nitrogen to the top meniscus of the specimen column. This will cause a local cold spot at the wall which leads to ice nucleation
- Lower the specimens back into the cryochamber and replace the cryochamber lid
- If necessary, raise specimens after 1 minute to confirm that the ice front is progressing
- Allow at least 10 minutes extra at the seeding temperature for latent heat to be absorbed and the first stage of dehydration to reach its equilibrium
- Continue the cooling protocol



KEY FACTS FOR MANUAL SEEDING

- The seeding location should be away from the embryo to avoid cells being frozen.
- Seed at the top meniscus of the column with the specimens.
- Ensure that only the upper meniscus of the specimen column is exposed when being raised. This guarantees that the sample is not exposed to temperature fluctuations.
- Specimens can be seeded individually or they can be raised collectively with the Lifter and seeded.
- If there is concern about self-seeding, use a higher seeding temperature.

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Innovative Instrumentation for Medicine & Life Sciences

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