

Thawing Cryopreserved Cells

During cryopreservation cells must survive a dramatic change in their environment, which may subject them to a variety of physical challenges, including the formation of damaging ice crystals and steep osmotic gradients.

With a quality cryoprotectant and detailed attention to the appropriate protocol, it is possible to cryopreserve cells with negligible loss of viability. However the thawing process is equally critical, as mishandling at this stage can easily destroy a vial of cells with otherwise excellent viability.

The Key Features of Good Thawing

During cryopreservation, the rate of temperature change must find suitable middle ground, to avoid the additional stresses created by rates that are either too fast, or too slow.

In contrast, is generally beneficial to thaw cells quickly, to reduce the exposure to potentially damaging ice crystals when the vial is only partly thawed.

Optimal thawing is generally achieved using a 37°C water bath. Hold the vial in the water bath without submerging the cap, and gently swirl the tube as the ice melts.

Do not leave the vial unattended, as at this temperature thawing is normally complete within 1-2 minutes. It is critical that the vial is not warmed up above 4°C as the cryoprotectants quickly become toxic as the temperature increases. Remove the vial from the water bath when a few ice crystals remain, and continue swirling to complete the last of the thawing process.

Gentle Handling is Critical

Freshly thawed cells are particularly vulnerable to damage, and must be handled with particular care. Any centrifuging at this time should be as gentle as possible, ideally not more than 120g. Many protocols actually recommend to seed thawed cells directly, thus avoiding the centrifuge step completely.

Seeding

Cells immediately post thawing are particularly sensitive. They should be seeded into pre-warmed medium. To assist the cells get started, low seeding densities should be avoided.

If cells are seeded directly without centrifuging, then the volume of the vial must be diluted at least 10x to minimize toxicity that otherwise may result from the cryoprotectant.

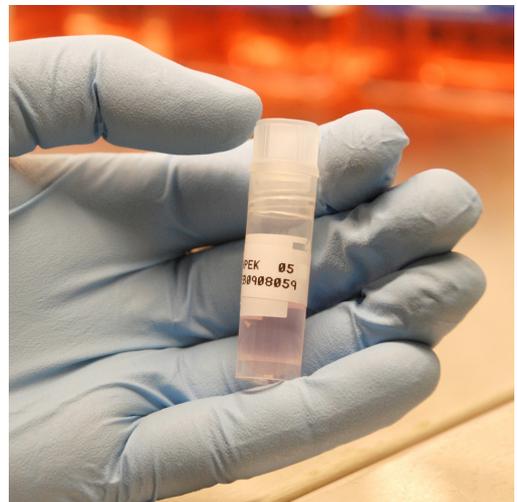
Then medium must be changed 6-24 hours later to remove any remnants of the cryopreservation medium.

See Also: Related knowledge-base article “Cryopreservation of Mammalian Cells”.

Questions about cryopreservation? Ask our scientists: scientist@cellntec.com

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Cryopreserved cells must be thawed quickly, without warming above 4°C.