Cryopreservation

Cryopreservation is a routine process in virtually all cell culture applications. Once stored in liquid nitrogen, cells can be maintained for extended periods (→ 10 years) without decrease in viability.

Cryopreservation of cells is not technically demanding, but does require very precise handling to ensure that the viability of the cells is not significantly compromised during the massive change in their environment.

Methods of Cryopreservation

Two primary methods of cryopreservation exist, namely slow freezing and vitrification. In slow freezing, cells are cooled over several hours (typically at a rate of approximately 1°C/min), which allows gradual equilibration of osmotic/salt gradients in and around the cell.

In contrast vitrification uses extremely rapid cooling, typically involving a direct transfer into liquid nitrogen (→20'000°C/min). When used with concentrated cryoprotectants that are also highly dehydrating, this method bypasses the formation of ice crystals completely, which is a major risk-factor associated with conventional slow-freezing methods.

Deadly Ice Crystals

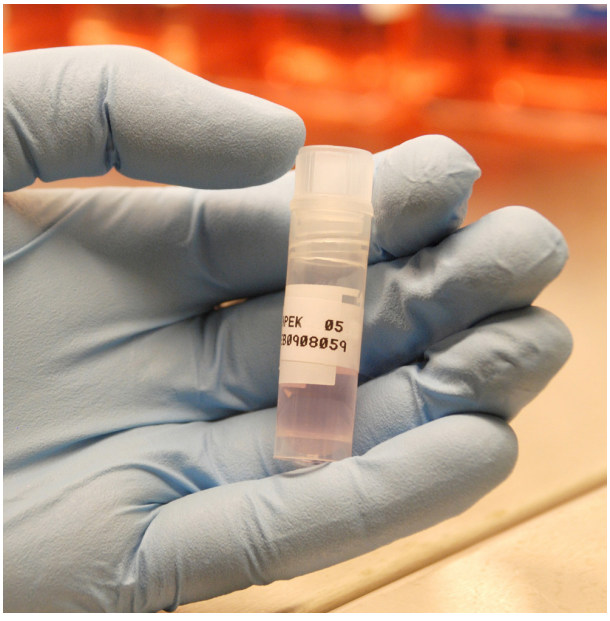
Formation of ice crystals in the cytoplasm during freezing will almost certainly rupture the cell membrane and kill the cell. To avoid this, low molecular weight cryoprotectant solutions (such as DMSO or ethylene glycol) are applied to the cells prior to freezing, where they penetrate into the cytoplasm and drive out the majority of the water.

Increased viscosity of the cryoprotectant can also aid cellular dehydration, and retard the spreading of any extracellular ice crystals that may form. With an optimal freezing medium and method, any remaining extracellular water condenses into small micro-drops in which the ice crystals form, leaving the cells frozen elsewhere in the now highly viscous, ice-free cryoprotectant.

Cryopreservation Protocols: Critical Details

The exact physical characteristics of a cryoprotectant dictate the optimal protocol for maximizing cell viability. Accordingly, each of the many kinds of cryoprotectants has its own optimal protocol. That said, there are several critical elements which must be carefully considered in most cryopreservation methods.

Adding the cryoprotectant/freezing medium. All cryoprotectants are designed to dehydrate, and are to some degree toxic. To avoid osmotic shock, they must be added gradually whilst swirling the tube. In parallel the temperature of all solutions must be kept close to freezing to avoid toxicity. Finally, cells should be left to sit on ice for 5 minutes after addition of the cryoprotectant, to allow penetration and osmotic equilibration.



The cryopreservation of mammalian cells requires close attention to subtle prototocol details.

Cooling rate. Optimal rates in the literature can vary significantly between cell types. However it is regularly reported that a cooling rate of 1°C/min is a good rate for many mammalian cell types. This rate generally provides a good balance between ice formation and osmotic stress (very rapid cooling increases the chance of intracellular ice formation, whereas slow cooling increases the osmotic imbalance across the cell membrane).

Transfer to liquid nitrogen. After controlled cooling to at least -70°C, cells should be transferred to liquid nitrogen. Although cells may be stored at -80°C without significant loss of viability in the short term (e.g. up to a week), only at temperatures below -130°C can genuine long-term storage be successfully achieved.

Liquid nitrogen storage. Vials may be stored either submerged, or in liquid nitrogen vapor. Submerged storage provides the most even temperature, but has an increased contamination risk (e.g. bacteria in the liquid). Conversely vapor storage tanks may have a noticeable temperature gradient; the amount of liquid in the bottom of the tank must be adjusted so that the temperature at the top of the tank is always below -140°C.

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

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