

Troubleshooting: Thawing Cryopreserved Cells

In general, cells should be frozen gradually and thawed rapidly. If problems occur try the following suggestions.

Problem	Possible Causes	Suggestions
Low Viability	• Cell density was too low when frozen	A certain amount of cell death can be expected so the concentration of cells should be high enough to take this into account. Start with a concentration of 10^6 to 10^7 cell/mL.
	• Problem during the thawing process	Frozen cultures should be stored in liquid nitrogen vapor or at -70°C to -80°C for 1-5 days, though this is not the preferred method of storage. Initiate the culture as soon as possible after thawing.
	• Sensitivity to the cryopreservative	Fully or partially replace the culture medium to reduce the amount of cryopreservative still present in the medium. Fluid changing after 24 hours will also expedite the removal of the cryoperservative. Allow the culture more time to recover. Sometimes it may take a few weeks for the cells to form a monolayer or dense suspension, depending on the age and passage number of the cells when frozen or position in the growth phase. Optimum conditions to freeze are when cells are in the log phase.
	• Age of frozen stock or age of culture at time of freeze.	Thaw a more recent freeze. The longer cells remain in the frozen state, the less viable they become. Check on the time and method taken to freeze cells. Cells should be in log phase at the time of freezing.
Large amounts of cell debris	• Cell lysis during thawing process	Decrease the time involved in the thawing process. Immediately immerse the vial containing in 37°C water bath and agitate until cells are about 75% thawed, then transfer to pre-warmed culture media.
	• Improper freezing process	Try thawing a vial from a different freeze. Ensure that proper technique was followed during the cryopreservation process. Check on amount of cryoperservative used.
Slow growth	• Size of culture flask	Some cells prefer to be close to each other in culture. Try transferring the culture to smaller flasks until cell density increases; e.g. from a 75cm^2 flask to 2 or 3 25cm^2 flasks, depending on culture volume.
	• Cell density too low	Increase the concentration of cells in future freezes.