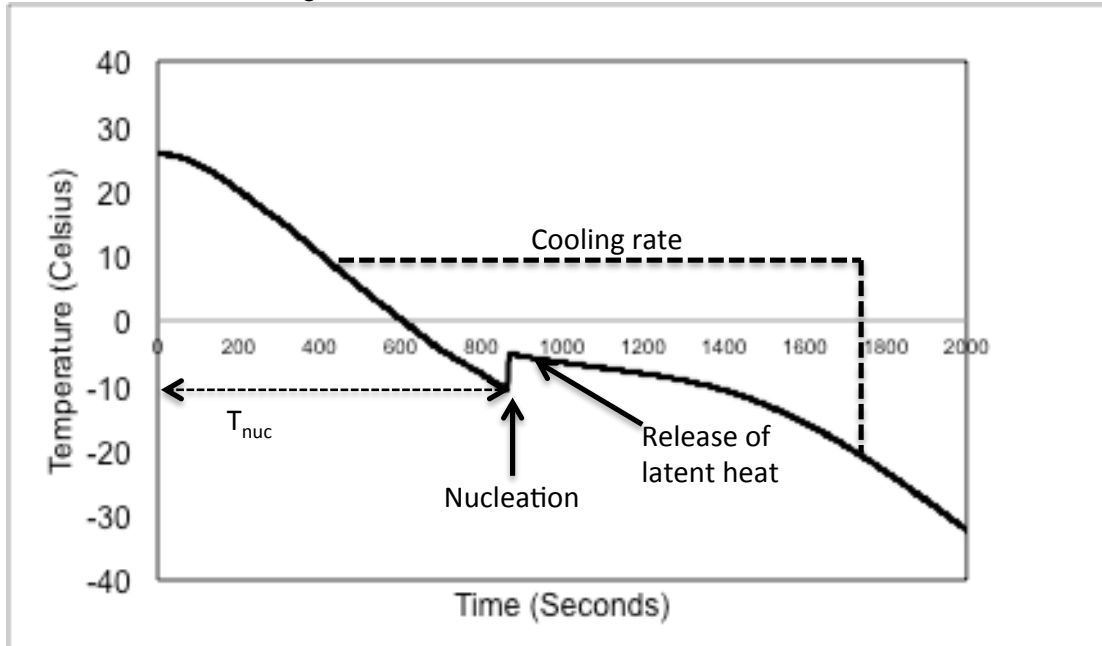


## Understanding your freezing curves 2Dec11

The rate of cooling (change in temperature as a function of time) influences post thaw viability for molecules, cell and tissues. For high value biospecimens (such as cell therapy products), it is common to monitor and record the temperature as a function of time during the freezing process as a form of process monitoring. Freezing curves become part of the processing documentation for the product and changes in the expected freezing curve may be considered deviations in the product processing. The purpose of this mini-tutorial is to describe critical elements in the freezing curve.



The first phase of the freezing curve is cooling of the sample from initial conditions to temperatures at which ice forms in the extracellular sample. The chemical environment of the sample does not change until the ice forms in the extracellular solution. At that point, water is removed in the form of ice and the unfrozen fraction of solution becomes more and more concentrated as freezing progresses. There is typically an increase in temperature immediately after nucleation and this corresponds to the release of the latent heat of fusion. When a protocol lists a cooling rate of 1°C/min, that cooling rate corresponds to the slope of the temperature versus time plot in the region shortly before nucleation through the freezing process to roughly -50°C (end temperature can vary with system. Some biological systems exhibit freezing response to considerably lower temperatures). This is the temperature range of greatest biological activity and this is the region over which knowledge of or control over the cooling rate is most critical. All of these events are marked on the accompanying figure.

Studies have shown in several different cell types that the lower the  $T_{nuc}$  (for a given cooling rate and composition of solution), the lower the post-thaw survival. When evaluating a freezing curve for deviation from expected norms, it is critical to determine  $T_{nuc}$  and determine if that actual value is lower than desired. Simply a delay in the time at which nucleation takes place does not necessarily correlate with reduced viability. It is the temperature at which the nucleation event takes place that is critical.

Keep in mind that nucleation is a stochastic event. That means that if you had a perfect controlled rate freezing (no spatial gradients in temperature) with 100 vials of the same solution being cooled in that freezer and each of those vials instrumented with a temperature measuring device, there would be a distribution of temperatures at which nucleation takes place. Most controlled cooling rate programs include a rapid decrease in temperature and then increase near the desired nucleation temperature (sometimes called automatic seeding or seeding step). The purpose of this step is to narrow the distribution of nucleation temperatures in products that are frozen. Unless you nucleate manually each sample that is frozen (done typically for gamete freezing), you cannot eliminate variations in the nucleation temperature between products completely-it is a function of the nucleation process. Passive freezing devices do not control the temperature at which nucleation takes place. Some cell types tolerate larger variations in nucleation temperature than others. Those that can tolerate large temperature differences will tolerate passive freezing.