Controlled rate freezing of stem cells: the importance of profiles.
The process

• Cell are preserved at cryogenic temperatures; typically -80 °C or -196 °C.

• Biological activity is slowed or stopped at these ultra-low temperatures.

• The controlled-rate freezer is used to get the cells down to the cryopreservation temperature without damage.
What occurs during preservation?

- At the start, the cell is surrounded by cryoprotectant and water.
- Penetrating cryoprotectants diffuse into the cell.
Ice starts to form

- Below the freezing point, extracellular ice forms if nucleated.
Latent heat is released

- When extracellular ice starts to form, there is a release of latent heat.
Concentration increases

- The formation of ice causes the solute concentration to increase in the remaining liquid.
- This depresses the freezing point further.
The cell begins to dehydrate

- The increased concentration of solute draws water out of the cell by the increased osmotic pressure.
The cell can be safely cooled

- Finally the cell is sufficiently dehydrated to prevent the formation of lethal intracellular ice.
But how fast?

- Cool too slowly:
  - Cells are damaged by long exposure to damaging concentrations of solutes.

- Cool too quickly:
  - Lethal intracellular ice is formed.

- Once sufficiently dehydrated, the cells can be rapidly cooled to the final storage temperature.
Latent heat release

- The point at which freezing actually starts varies between samples.
- It depends on the number of nucleation sites.
Risk of intracellular ice

- The chance of intracellular ice forming is increased if freezing starts later.

LARGE RISE.
FAST RETURN TO PROFILE.
Repeatability

- Inconsistency in the release of latent heat leads to a loss of repeatability.
- Without intervention, the start of ice formation depends on the presence of nucleation sites.
- The further below the freezing point, the greater the probability of ice forming.
- How can we ensure repeatable results?
Seeding dip

- By introducing a dip, ice nucleation can be initiated in a consistent and repeatable manner.
Example run with a seeding dip

- In this example the user has programmed the freezer to move to next step when the sample has reached the end temperature. This is indicated in the trigger column.

<table>
<thead>
<tr>
<th>Rate °C/min</th>
<th>End temperature °C</th>
<th>Trigger</th>
</tr>
</thead>
<tbody>
<tr>
<td>-2.0</td>
<td>-4</td>
<td>sample</td>
</tr>
<tr>
<td>-35.0</td>
<td>-60</td>
<td>chamber</td>
</tr>
<tr>
<td>-8.0</td>
<td>-20</td>
<td>chamber</td>
</tr>
<tr>
<td>-2.5</td>
<td>-45</td>
<td>chamber</td>
</tr>
<tr>
<td>-10.0</td>
<td>-80</td>
<td>sample</td>
</tr>
</tbody>
</table>
Recap

- Cryopreservation in a controlled-rate freezer removes water from the cells.

- A seeding dip can improve repeatability by initiating ice nucleation.

- Further sources of information:
The end.
For further information contact sales@planer.com

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