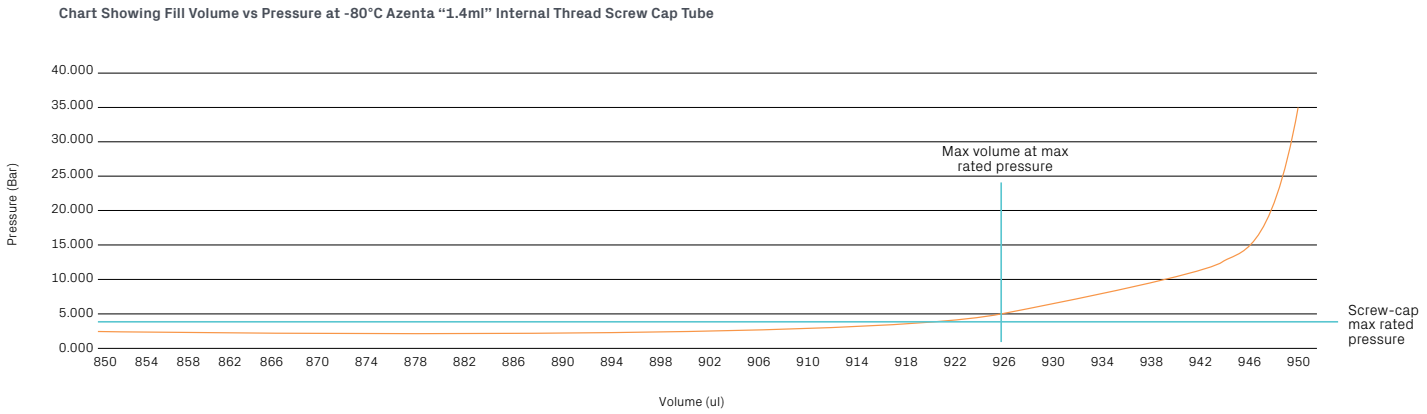
Cryopreservation: Azenta Freezing Protocols

Introduction

Freezing of biological samples is the most common form of preservation. In general temperatures of -20°C, -80°C and -196°C (Vapor phase liquid nitrogen) are used.

There are many studies examining the best freezing protocols that can be used to enhance the preservation of active biochemicals and whole cells. However this technical note focuses on the effect freezing may have on a sealed sample tube and measures that should be taken to prevent undue stress or damage to the tube.

On freezing water expands by approximately 9% and in a sealed system this expansion causes an increase in internal pressure. The rise of pressure can be largely accommodated for by leaving an adequate "head-space" of air above the sample. As the liquid expands the air easily compresses with little increase in internal pressure. However there are two circumstances where this may not prevent a dramatic rise in internal pressure resulting in significant damage to the tube.



Over-filling

If the tube is over-filled the head-space may be too small to accommodate the liquid expansion. Once the air is compressed the internal pressure will rise very rapidly with only a very small increase in volume. The chart above demonstrates this by showing a rise in internal pressure of 5 bar by exceeding the maximum volume by only 20% or less than 2.5% of the total volume; within the accuracy limits of some liquid handling systems.

Ice Plugs

Regardless of fill volume it is possible that an ice plug can form at the top of the tube leaving liquid in what is then an effectivity sealed tube. As this remaining liquid freezes the pressure can rise to levels that cause damage to the tube. This is exactly the same phenomenon as seen in domestic pipes when ice-plugs cause burst pipes.

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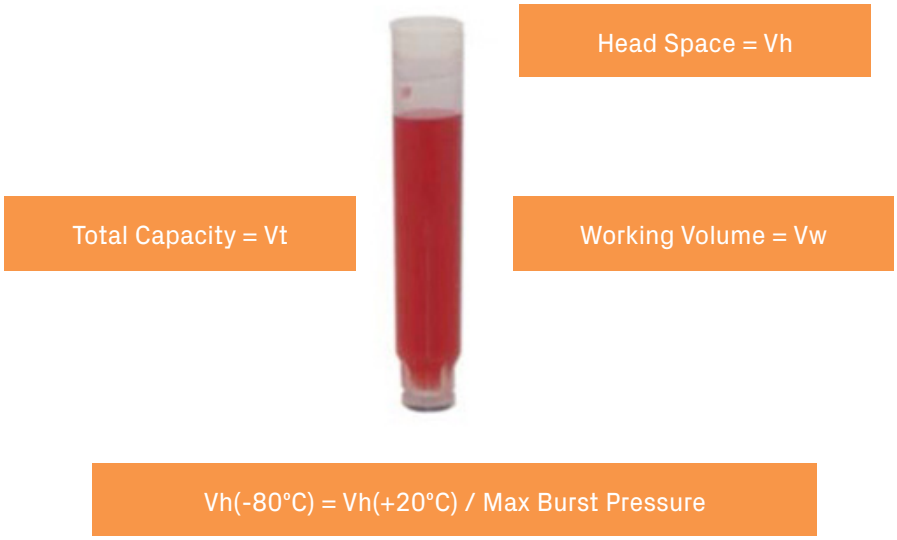
Our Method

Over-filling

Azenta has calculated the maximum filling volume for all of our tubes using the method below. Combined with safe maximum pressures measured for every tube by experiment allow us to provide a safe maximum working volume.



Ice Plugs

Ice plugs are generally only ever seen when using (i) larger tubes of 4ml volume and above and (ii) when using a tube and rack combination where the tube protrudes significantly above the rack where the tube is exposed to a greater cooling rate than the base of the tube. Our range of 96 format tubes in SBS racks match tube and rack to avoid this issue.

All Azenta stores provide a controlled rate freezing environment that eliminates the opportunity for ice plugs to form. Additionally, all Azenta cryovials are made from homopolymerpolypropylene (rather than the commonly used copolymer polypropylene) because of its greater tensile strength and temperature resistance.

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