

Thawing Recommendations

for Successful Outcomes

Sample Filling

1. Samples should be filled to the recommended limits of the cryogenic freezing bag (cryobag) manufacturers. Overfilled or underfilled bags increase the probability for sensor qualification issues.
2. Air pockets in the cryobag should be minimized during sample filling. Air bubbles in the cryobag may cause uneven sample warming during thawing, which may cause temperature detection errors.
3. Sample preparation for cryopreservation is recommended to use CryoStor® CS10 (<https://bit.ly/3jHD9YT>) or other cryopreservation media without animal products or serum. Optimized thawing methods are aligned with serum-free and protein-free cryopreservation media, such as CryoStor CS10. Visual outcomes of thawed samples may differ depending on cryomedia formulations.

Note: Optimization of ThawSTAR CB profiles is provided as a service by BioLife Solutions technicians that may provide benefit for cryomedia that contain different amounts of Dimethyl Sulfoxide (DMSO) or other cryoprotectants.

Sample Bag and Freezing Cassette

4. Using an appropriate freezing cassette with the cryobag to ensure even sample distribution is recommended. Place the cassette containing the cryobag in a horizontal position when freezing. During freezing the cryobag access ports should be oriented as shown in **Figure 1**.
5. To help ensure a flat even distribution of media in the cryobag, a felt insert may be added into the cassette. The felt insert may vary in size depending on the size and fill of the cryobag. The felt insert is placed between the cryobag and the cassette prior to freezing to cause the cryobag to freeze flat when the cryobag ports are oriented appropriately. Flattening of the cryobag allows for greater temperature distribution and ease of thawing on the ThawSTAR CB. (**Figure 2**)

Figure 1



Figure 2



6. For 50ml cryobags that have a flap extension feature, the flap should be on the topmost side during freezing as shown in **Figure 3**.
7. It is recommended that the sample is a flat, even, frozen cryobag, for optimal thawing with ThawSTAR CB. (**Figure 4**)

Frozen Bag Storage

8. Samples that will be stored in Liquid Nitrogen (LN2) dewars may be stored vertically or horizontally once the samples are frozen.

Frozen Sample Removal from Storage

9. Prior to removal of the frozen cryobag from LN2 storage, start the ThawSTAR CB and allow enough time for the machine to complete preheating sequence.
10. To reduce the effect of passive, uncontrolled heating during transit of the cryobag, begin the thawing process by following the ThawSTAR CB on-screen instructions and profile selection prior to sample removal from storage.
11. Use appropriate Personal Protective Equipment (PPE) per customer internal Standard Operating Procedures (SOPs) to remove cryobag.
12. Insert cryobag into the barrier bag per on-screen instructions.

Following ThawSTAR CB Thawing Completion

13. Following completion of the thaw there should be a slush mixture in the cryobag. After removal, knead the thawed cryobag using gloved hands for ~10-20 seconds or until ice is completely melted.

Figure 3



Figure 4

