

# How to Prevent Freezing Bag Breaks

Recommendations for bag handling  
to reduce incidence of bag breakage.

# HOW TO PREVENT FREEZING BAG BREAKS

All freezing bags can be broken while frozen, - EVA, EVO, FEP, every flexible bag material can be broken when frozen solid. Of course, some materials are more rugged than others, but with a bit of care, you can prevent breaks altogether. Of the few breakage complaints we receive, almost all come from relatively new customers. Once they implement the changes below, their breakage problem disappears.

*Here is what we recommend:*

- Seal Donor Tubes Short
- Remove Air
- Use the Right Cassette
- Handle with Care
- Use the Right Cryoprotectants
- Be Aware of Cell Metabolism

## SEAL DONOR TUBES SHORT

This is the most common cause we see contributing to bag breaks. The donor tube should be sealed below the spike ports and as close to the stub tube as possible (see photo at right). Sealing it short protects the frozen donor tube from potential impact while frozen if the bag slides within the cassette. Conversely, leaving it long makes it act like a lever, and any impact on the frozen tube can snap it off at the bag joint. Finally, cut, don't pull, the seal apart. This may work for PVC, but not for EVA tubing used on the Cryostore bag.



## REMOVE AIR

Air is made of 79% nitrogen, which will be liquefied when stored in LN. This liquid then runs down to collect at the lowest open area in the bag. The problem is that during re-warming, nitrogen expansion back to the gas phase will occur while the bag is still brittle, and most likely in different regions than where it was before liquefaction. The resulting rapid expansion can create a bubble of pressure and cause a crack or bursting of the bag.

## USE THE RIGHT CASSETTE

If the storage cassette is too tight or has projections that distort the bag, that is a problem. The bag expands as it warms, and the chance of the bag shifting to a different area during storage is high. As the bag expands into a restricted space, a pinch can result in a bag break. At the same time, if the cassette is too large, the frozen bag can slide around in the cassette, and the impact of sliding into the metal cassette can cause a crack. Use of the OriGen Overwrap bag may help keep the bag protected and in place.

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## HANDLE WITH CARE

If you handle the bag like it is fragile when frozen, your extra care will result in fewer bag breaks. On the other hand, if you transport the frozen bag rattling around loose in a hard container as you push it across the parking lot, you should not be surprised to find a break. Bag breaks that develop during freezing and/or thawing occur due to impact, crushing, or bending of the frozen CryoStore bag. When frozen below  $-60^{\circ}\text{C}$ , the EVA acts like a pane of glass and can crack if enough force is applied. Use care when removing, transporting, re-racking, and thawing all frozen bags.

## USE THE RIGHT CRYOPROTECTANTS

Different cell cultures and different cryoprotectants can behave differently and actually expand during freezing. Water goes through several different phase changes on its way down to LN temperatures, some of those phase changes expand dramatically. We all know icebergs float, so we shouldn't be surprised to see expansion happen during freezing. Know what to expect from your solutions and maintain consistency in your processing.

## CELL METABOLISM

This is the one that is trickiest of all, and the most maddening to figure out. If your cells are actively growing just before freezing, they are expelling a lot of  $\text{CO}_2$ . Carbon Dioxide liquefies, seeks the lowest level, and then freezes at  $-78^{\circ}\text{C}$  ("Dry Ice"). The volume decreases dramatically as it goes from gas to solid. As the bag is subsequently warmed above  $-78^{\circ}\text{C}$ , the solid  $\text{CO}_2$  formed during cryopreservation goes suddenly to gas, and increases in volume about 1000 times. Since it has likely trickled to a new location, it cannot expand back into its original area. The gas pressure inside the bag causes it to crack if still frozen, or explode with a 'POP' if the bag material is warmed. We have seen both happen.

We believe that if you are aware of these few safety measures and use care in handling frozen cells and tissues, you will join the hundreds of users who have never had a bag break.