

General Use Guidelines for KryoSure® Bags

KryoSure® Bags are exclusively designed as standard cryopreservation containers. These bags are used for closed system sterile transfer and aseptic recovery of sterile contents such as cellular vaccines, donor lymphocyte infusions, and other clinical and industrial applications.

This bag provides the ability to freeze, thaw, and dispense individual doses created from a common reservoir.

- The bag contains and protects liquids and suspensions in mechanical freezers, vapor phase, and liquid nitrogen environments.
- KryoSure® Bags are made of FEP film, remaining flexible at -200°C to +200°C.

CRYOPRESERVATION GUIDELINES FOR CELLS TO BE STORED IN KRYOSURE® BAGS

**Note: Perform all of the following steps according to the safety standards set by your institution.*

Note: These steps outline a **manual procedure for cryopreserving cells in KryoSure® Bags. In a fully closed system, users can use sterile tube welding devices to transfer cells.*

1. Collect cell population to be cryopreserved (e.g. harvest cultured cells from a VueLife® bag) in an appropriate transfer vessel (e.g. conical tube).
2. Determine cell viability and number of cells (this step is traditionally done using the Trypan Blue Dye exclusion method and a Cell Analyzer, but other methods and instruments can be used as preferred by the user).
3. Following gentle centrifugation of the cells in a conical tube (typically 130 X g for 10 minutes), discard the supernatant and gently re-suspend the cells in an appropriate amount of Cryopreservation Medium containing cryoprotectant.

Manual Filling of the KryoSure® Bag

4a. Transfer the cell suspension using a sterile syringe and needle (typically a 16g or 18g syringe is used). Carefully pull the cell suspension into the syringe and safely cap the needle.

4b. Remove the KryoSure® Bag from its sterility pouch. Typically, the port with the female luer-lock is used for filling. For the smaller 6-F KryoSure® Bags, the FLV port is used.

4c. Safely remove the needle from the end of the filled syringe. Safely attach the male luer end of the syringe into the female end port attached to the PVC tubing.

4d. Slowly inject the cell suspension into the KryoSure® Bag and **remove any excess air in the bag using the attached syringe**. Air removal and proper bag filling is essential for optimal freezing. The cell suspension should completely fill the bag in a thin layer. Remove the syringe and re-cap the port.



Fig. 1 Filling a KryoSure® Bag using a syringe

**Note: Please see website for bag sizes and recommended fill volumes. <https://www.celltherapy.saint-gobain.com/products/kryosure-cryopreservation-bags>*

Closed System Filling of KryoSure® Bags

5. The cell suspension can be transferred from a bag to the KryoSure® Bag using sterile tubing welders to create a sterile dock. Once the bags are sterilely connected, the contents of the bag can be gravity fed into the KryoSure® Bag.
6. Seal off/cut any long lengths of tubing using a sterile tubing sealer/welder. If possible, roll the excess tubing into the available protective pocket flaps before sealing the FEP pocket flaps closed.

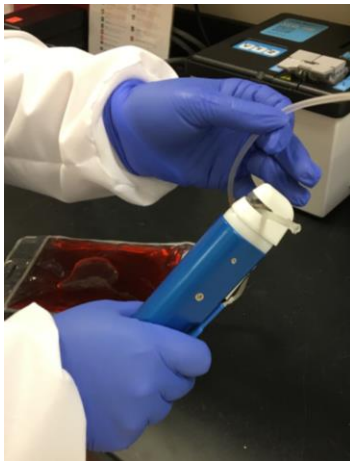


Fig. 2 Cutting and sealing of tube

7. KryoSure® Bags are designed with an ISBT 128 compliant label pocket. By special order, serial numbers can be engraved into the label pocket and etched into the fill tube in three places providing unalterable segments for cross-identification. Additionally, RFID tags and/or printed labels (that are recommended for use with FEP and extreme temperatures) can be used with this pocket.

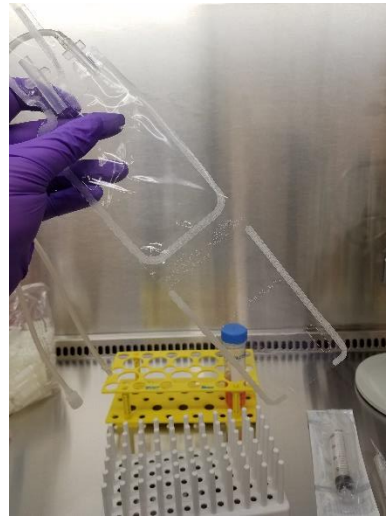


Fig. 3 KryoSure® Bag with label pocket

8. Place the bag into the appropriate sized metal cassette or KryoSure® Overwrap and into the freezer (ideally, a controlled-rate freezer) to bring down the temperature to the first set point (often -80°C).

**Note: When placing bags in the metal cassette, try to insure that there are minimal folds, bends, and stresses on the bag/tubing.*



Fig. 4 (left) KryoSure® Bag assembly contained in overwrap (right) KryoSure® Bag in cassette after freezing

9. Place the bag/cassette into the designated cassette rack and into the cryofreezer. Cool down to desired temperature for cold-storage.

THAWING GUIDELINES FOR CELLS STORED IN KRYOSURE® BAGS

**Note: If bags are stored in liquid nitrogen or vapor phase, we suggest the following steps be done in accordance with appropriate Liquid Nitrogen Use Protocols and PPE, and safety standards as mandated by your institution.*

1. Carefully remove the racks and cassettes storing the KryoSure® Bag from the cryofreezer.

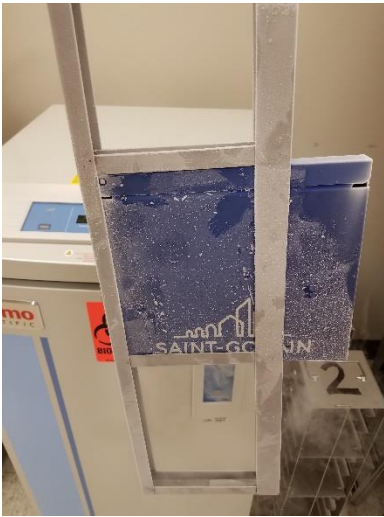


Fig. 5 Cassette being removed from liquid nitrogen freezer

2. Remove the cassettes from the rack and carefully open the cassette. Inspect the KryoSure® Bags and tubing for integrity.
3. Place the KryoSure® Bag in an overwrap (if it was not already stored in one) and add the bags to a heated (37° C) water bath to quickly thaw. Monitor the temperature of your water bath for an efficient thawing process.



Fig. 6 KryoSure® Bag being placed in water bath

4. Once the majority of the contents are visibly thawing, remove the KryoSure® Bag from the water bath. Disinfect the outside of the bag prior to further processing.
5. When ready to retrieve contents, tear off the FEP port protector at the indicated tear initiator.



Fig. 7 Female spike port with protective port pocket and tear away section for use after thawing

- Depending on the type of port available, a male sterile spike may need to be attached to the newly exposed female port.

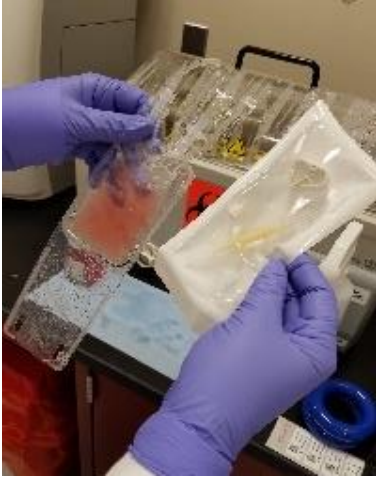


Fig. 8a (Left) KryoSure® Bag (right) male spike port to be used with female port



Fig. 8b Insertion of male spike into female spike port

- Extract the thawed contents using the appropriately sized syringe and needle (if necessary). When working in a closed system, sterile tube docking is recommended. When it is possible, use different ports for addition and retrieval of bag contents.



Fig. 9 Contents being transferred using syringe

- Process the contents of the KryoSure® Bag as defined by your institution's procedures.

ABOUT

SAINT-GOBAIN

Saint-Gobain Life Sciences is proud to take part in providing solutions for a multitude of cell therapy applications while collaborating with customers and industry partners to develop custom disposables, often for integration into automated systems. Through our material science expertise as well as our deep experience in bringing manufacturing technologies to scale, we are uniquely positioned to offer solutions to the numerous challenges faced by cell therapy manufacturers today.