

Instructions for Preparing a Cord Blood Unit for Patient Infusion

Reference Document

The cord blood unit (CBU) is stored continuously inside a steel canister in liquid nitrogen at temperatures $\leq -150^{\circ}\text{C}$. For shipment, the canister is placed inside a container specifically designed to keep the temperature at or below -150°C (dry shipper). It is recommended to keep the canister inside the dry shipper for short-term storage (up to 48 hours) or transfer it into a liquid nitrogen (LN₂)-cooled storage device at the Transplant Center for storage greater than 48 hours.

I. MATERIALS

Equipment:

- Automated cell counter and/or microscope and cell count chamber for cell count and viability determination (optional)
- Biological safety cabinet (BSC)
- Canister-opening tool (supplied by LifeSouth Community Blood Centers)
- LN₂ storage freezer at -150°C or colder
- Plasma extractor/expressor
- Refrigerated centrifuge
- Scale
- Sterile docker
- Tube sealer compatible with polyvinyl chloride plastic
- Water bath

Personal Protective Equipment:

- Closed-toe shoes
- Cryo-protective gloves
- Lab coat
- Latex or non-latex gloves (sterile preferred)
- Safety glasses or face shield

Reagents:

- Dextran 40 (10% LMD) (stored at 2 to 6°C)
- 5% Albumin (human) solution (stored at 2 to 6°C)

Supplies:

- 30-mL sterile syringes
- 60-mL sterile syringes
- 300-mL or larger transfer packs
- Alcohol pads
- CBU
- Disinfectant solution
- Hemostats
- Needle-free luer locks
- Needles
- Paper towels
- Refrigerated gel packs
- Sampling site couplers
- Scissors
- Small sterile, resealable plastic bags
- Sterile sampling cups, pipettes, and syringes if necessary to perform emergency recovery procedure
- Sterile water for water bath
- Timer

Forms:

- *Umbilical Cord Blood Cryopreserved Unit Receipt Instructions*
- *Umbilical Cord Blood Cryopreserved Transfer Report*

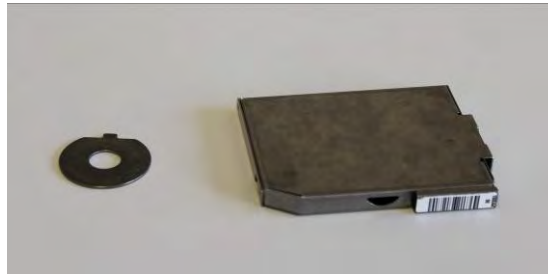
II. PROCEDURE NOTES

- Handle the frozen cord blood bag with extreme care at every step, including opening the metal containers, inspecting, thawing and/or washing.
- Use standard procedures and competent personnel to perform post-thaw sampling and/or bag recovery.
- Perform all steps on lab benches, under biological safety cabinet, or another surface to prevent inadvertent drop of the frozen unit.
- Put the frozen bag inside a resealable plastic prior to initiating the thaw to facilitate salvage of the product and to reduce the possibility of contamination.
- If the CBU is seen to be cracked when removed from the LN2 storage container, or if cracks or leaks occur during thawing, immediately notify LifeSouth at 1-888-795-2707. Notify the transplant physician/team and the laboratory director as soon as possible.

III. PRODUCT IDENTITY VERIFICATION

1. Apply personal protective equipment.
2. Open the dry shipper lid upon receipt.
3. Verify that the National Marrow Donor Program (NMDP) number on the *Umbilical Cord Blood Cryopreserved Transfer Report* matches the NMDP number on the CBU. If the NMDP numbers do not match, contact LifeSouth Community Blood Centers at 1-888-795-2707.
4. Remove the canister from the dry shipper and the canister-opening tool from the shipment documentation packet (see Figure 1).

Figure 1:

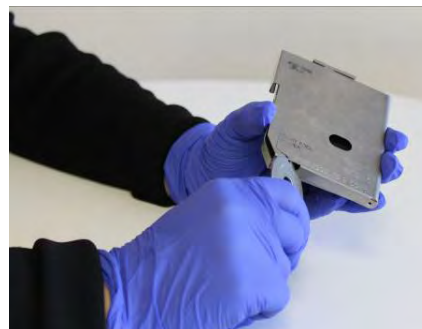


5. Compare the product barcode label located on the side of the canister (see Figure 1) with the product identification (ID) information included in the package. Verify this information as soon as the shipment arrives and before administering the CBU. If the barcoded label is not found on the outside of the canister, the product barcode information can be found on the frozen CBU enclosed in the canister.
6. Using cryo-protective gloves and the canister-opening tool, open the canister at top and bottom using the following steps to avoid damaging the frozen cord blood bag:
 - a. Align the canister-opening tool with the slot in the bottom of the canister (see Figure 2).
 - b. Turn the canister-opening tool clockwise to open the bottom of the canister (see Figure 3).
 - c. Align the canister-opening tool with the slot in the top of the canister (see Figure 4).
 - d. Turn the canister-opening tool counterclockwise to open the top of the canister (see Figure 4).

Figure 2:

Figure 3:

Figure 4:



- e. Open the canister hinges (see Figure 5).

Figure 5:



7. When the canister is open, compare the product barcode information with your records.
8. Close canister after verification is complete.
9. Using cryo-protective gloves, return the canister to the dry shipper for short-term storage (up to 48 hours) or to an LN₂ cooled storage device for storage greater than 48 hours.
10. When all records are verified, indicate acceptance by recording initials and date in the indicated space on the *Umbilical Cord Blood Cryopreserved Unit Receipt Instructions* and *Umbilical Cord Blood Cryopreserved Transfer Report* forms.


For Incorrect Information:

1. If any information is incorrect or cannot be verified, close the canister and return the frozen CBU to the dry shipper for short-term storage (up to 48 hours) or to an LN₂-cooled storage device for storage greater than 48 hours.
2. Immediately report the discrepancy to LifeSouth at 1-888-795-2707 and to the transplant physician.
3. Perform a thorough investigation, while keeping the CBU frozen at or below -150°C until all discrepancies are resolved.
4. When all records are verified, indicate acceptance by recording initials and date in the indicated space on the *Umbilical Cord Blood Cryopreserved Unit Receipt Instructions* and *Umbilical Cord Blood Cryopreserved Transfer Report* forms.

IV. PREPARATION

A. Prepare Thawing Solution

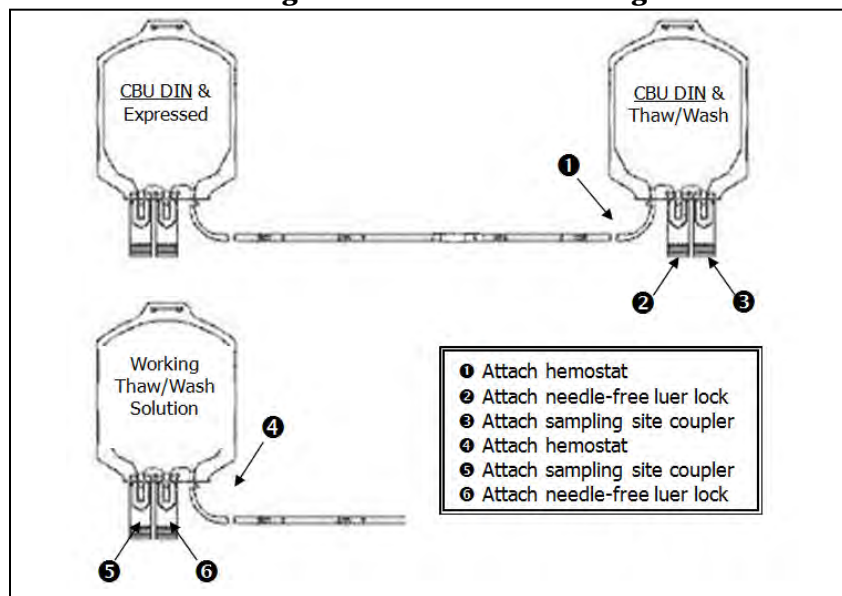
Note

 Prepare thawing solution at least one hour and no more than 24 hours before thawing the CBU.

1. Obtain two gel packs; place in refrigerator to cool.
2. Label a 300-mL or larger transfer pack with "Working Thaw/Wash Solution" and the date/time prepared (see Figure 6).
3. Perform the following steps under a biological safety cabinet to prepare the thaw/wash kit:
 - a. Clean ports with alcohol, and attach a sampling site coupler to one port and a needle-free luer lock to the other port; attach hemostat to tubing (see Figure 6).
 - b. Add at least 112.5-mL Dextran to the 300-mL transfer pack labeled Working Thaw/Wash Solution using 60-mL sterile syringes with needles (Albumin to Dextran ratio must be 1:1).
 - c. Add at least 112.5-mL 5% albumin (human) solution to the same the 300-mL transfer pack labeled "Working Thaw/Wash Solution" using 60-mL sterile syringes with needles.

- d. Mix by inverting the pack at least 10 times.
- e. Label one transfer pack with CBU Donation Identification Number (DIN) and "Thaw/Wash." Clean ports with alcohol, and attach a sampling site coupler to one port and a needle-free luer lock to the other port (see Figure 6).
- f. Label the second transfer pack with CBU DIN and "Expressed."
- g. Attach a hemostat to the tubing approximately one inch above the sampling site coupler on the CBU DIN and Thaw/Wash transfer pack (see see Figure 6).
- h. Using a sterile docker, connect the two 300-mL transfer packs (see see Figure 6).
- i. Add 100 mL of thaw/wash solution to the CBU DIN and Thaw/Wash transfer pack.
- j. Label two 60-mL sterile syringes and two 30-mL sterile syringes as "Thaw/Wash Solution."
 - i. Draw 20 mL of thaw/wash solution into each of the two 60-mL syringes; cap and set aside.
 - ii. Draw 5 mL of thaw/wash solution into each of the two 30-mL syringes; cap and set aside.
- k. Label a 60-mL sterile syringe "Resuspension Solution"; fill with 50 mL of pre-chilled thaw solution (1:1 ratio of Dextran/albumin (human) solution) for final end-product resuspension via needle-free luer lock. Cap and place in refrigerator to use after centrifugation.
- l. Place the transfer packs and syringes in the refrigerator for at least one hour and no longer than 24 hours.

Figure 6: Transfer Pack Diagram



B. Thaw CBU

Schedule the transplant infusion time with the transplant team prior to performing the procedure. Reconfirm with the transplant team on the day of infusion so the start time for the thawing procedure can be adjusted as necessary in order to have the unit ready for infusion at a time the patient can receive the infusion.

Prepare a water bath using sterile water; allow water bath to equilibrate to a temperature of $37\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ prior to retrieving CBU for thaw.

If canister is stored in liquid phase LN_2 , wear cryo-protective gloves to lift the canister containing the CBU from the liquid phase of the LN_2 container, and rest canister in the vapor phase within the container for five to ten minutes before proceeding.

Note

☛ *Carefully check the identity of the unit to be thawed.*

1. Open the canister with the canister-opening tool (refer to section **III. PRODUCT IDENTITY VERIFICATION**). Avoid damage to the plastic bag containing the frozen CBU. Carefully examine the plastic bag for breaks or cracks.

Note

☛ *Verify bag labels are intact, ports are unopened, and there is no visible damage throughout the remainder of thaw procedure.*

2. Remove the CBU from the canister and remove the overwrap; place in a sterile, resealable plastic bag, remove excess air, and close tightly.
3. Place the resealable plastic bag containing the frozen cord blood into water bath (see Figure 7). Thaw CBU until slushy and not completely thawed; watch ports closely to ensure ports are thawed. Watch closely for any cracks or breaks, as shown by red cells leaking from the CBU into the resealable plastic bag; minimize loss of CBU by positioning or pinching the CBU to prevent leakage into the resealable plastic bag.

Figure 7:



4. If leakage occurs, keep the entire pack upright to prevent further leaking until product is slushy and not completely thawed. Perform section **VI EMERGENCY RECOVERY PROCEDURE IN THE EVENT OF A CONTAINER FAILURE**.
5. If no leakage occurs, remove the resealable plastic bag from water bath. Dry the outside of the bag, disinfect it with alcohol, and place it inside a biological safety cabinet.

C. Add Thawed CBU to Transfer Pack

Complete the following steps inside a biological safety cabinet:

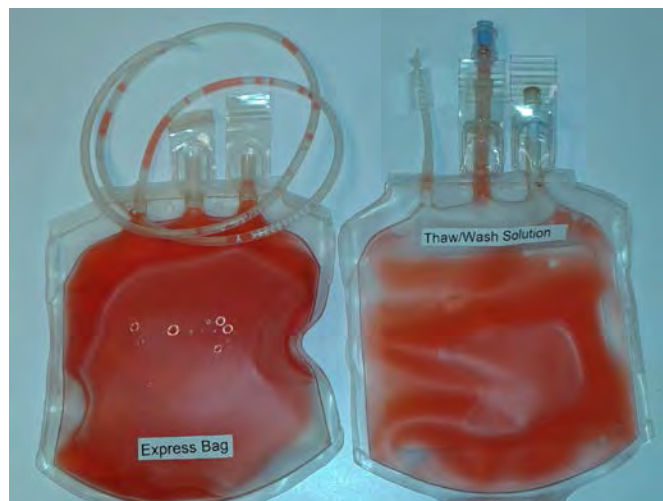
1. Obtain and disinfect materials before placing under BSC.
2. Remove two gel packs, two 30-mL syringes, and two 60-mL syringes from refrigerator; disinfect and place under BSC. Place absorbent material, such as a paper towel, on top of one of the gel packs.
3. Obtain CBU; disinfect, place under BSC, and remove from resealable plastic bag.
4. Obtain scissors; disinfect scissors and port covers, then cut off port covers.
5. Disinfect cut port-cover surfaces with alcohol and attach one needle-free luer lock to each port.
6. Obtain 30-mL syringe containing 5 mL of thaw/wash solution and insert into small compartment port; slowly inject thaw/wash solution into small compartment. Do not remove syringe.
7. Obtain 60-mL syringe containing 20 mL of thaw/wash solution and insert syringe into large compartment port; slowly inject thaw/wash solution into large compartment. Do not remove syringe.

8. Place CBU on refrigerated gel pack. Set timer for 5 minutes, and place second gel pack on top of CBU. When alarm sounds, remove CBU from gel packs.
9. Slowly pull back and push in the syringe plunger to mix the cord blood and thaw/wash solution; repeat until thoroughly mixed.
10. Draw all fluid from the compartments; inject into the CBU DIN and Thaw/Wash Solution transfer pack via sampling site coupler while slowly mixing Thaw/Wash Solution transfer pack.
11. Obtain second set of syringes, including a 30-mL syringe containing 5 mL of thaw/wash solution and up to 5 mL of air and a 60-mL syringe containing 20 mL of thaw/wash solution and up to 20 mL of air. Repeat steps 7, 9 and 10.
12. Mix the CBU DIN and Thaw/Wash Solution transfer pack well by inverting the transfer pack 180° about 10 to 15 times.
13. Discard remaining Working Thaw/Wash Solution.

D. Wash the Thawed CBU

1. Obtain thawed CBU in Thaw/Wash Solution transfer pack containing the hemostat, sampling site coupler, luer lock, and additional transfer pack; mix by inverting at least ten times. Place into a resealable plastic bag, and place in the refrigerated centrifuge in an upright position. Do not allow pack to crease.
2. Ensure the centrifuge is balanced before beginning centrifugation cycle.
3. Centrifuge at 400 g or 1200 rpms (rpms will be centrifuge specific) for 20 minutes at 10°C with no brake and slow stop.
4. After centrifugation, carefully remove the Thaw/Wash Solution transfer pack from the centrifuge and look for clear separation of supernatant from cell pellet.
5. Place transfer pack containing CBU and thaw/wash solution into a plasma extractor and allow supernatant to flow into second transfer pack labeled Expressed Solution by removing the hemostat from the tubing. Express as much supernatant as possible without sacrificing cells; the volume should be as low as possible to remove the DMSO and thaw waste without allowing the cell pellet to escape.
6. Hemostat tubing after expressing to close the tubing.
7. Place Thaw/Wash Solution transfer pack on a flat surface and gently massage the pellet to release the cells into suspension.
8. Heat seal tubing with spike on CBU DIN and thaw/wash solution closest to primary pack three times; ensure the first seal is approximately three inches away from the primary pack (see Figure 8). Cut the middle seal.

Figure 8:



9. Remove hemostat from tubing.
10. Weigh and calculate the volume of the Thaw/Wash Solution transfer pack.
11. Weigh and calculate the volume of the Expressed Solution transfer pack.

E. Resuspend and Sample the Thawed CBU

1. Obtain the prepared 60-mL syringe labeled "Resuspension Solution" from the refrigerator.
2. Clean the port with alcohol, and slowly add 50 mL of thaw solution to the Thaw/Wash transfer pack from the 60-mL syringe.
3. Mix Thaw/Wash transfer pack well by inverting the transfer pack 180° about 10 to 15 times.
4. Complete sampling for any necessary testing.
5. Label DIN and Thaw/Wash Solution transfer bag with the expiration time and time of wash completion. The recommended expiration time is 2 hours after the completion of wash until infusion, if stored at room temperature (19 to 25°C) or 4°C.
6. Notify the Transplant Center that the CBU is thawed, washed, and available for infusion.

V. ADMINISTRATIVE REQUIREMENTS

1. Prepare a written summary of the procedure, including:
 - a. CBU ID number
 - b. Date of receipt of CBU
 - c. Liquid nitrogen storage temperature
 - d. Date of thaw, including whether and at what stage leaks or cracks occurred
 - e. Date and time CBU removed from liquid nitrogen storage
 - f. Volume of final product
 - g. TNC (Total nucleated cell) count, CD34+ count
 - h. Viability of recovered cells (TNC or CD34+) plus name of method used
 - i. Results of bacterial and fungal cultures
2. Make a copy of the report for your records.
3. Fax a copy of the report to LifeSouth at (352) 334-7758.
4. Return the dry shipper to LifeSouth. The return address is:

LifeSouth Community Blood Centers, Inc.
LifeCord Cord Blood Bank
4039 Newberry Road
Gainesville, FL 32607

Phone: (888) 795-2707
Fax: (352) 334-7758

VI. EMERGENCY RECOVERY PROCEDURE IN THE EVENT OF A CONTAINER FAILURE

The transplant physician or team will determine whether to use or discard the CBU product and whether any additional units should be requested. If the transplant physician or team decides the leaking CBU can be used, recover the CBU as follows:

1. Obtain sterile sampling cups, pipettes, and syringes.
2. Open sterile sampling cups and arrange in workspace to receive contents of the resealable plastic bag and the cracked/leaking CBU.
3. If any contents remain in the CBU, remove using the syringes prepared in Section A. **Prepare Thawing Solution.** Wash all of the CBU contents and add to the CBU Thaw/Wash Solution transfer pack contained in the transfer set.
4. Using a sterile syringe, transfer 20 mL from the CBU Thaw/Wash Solution transfer pack into a sterile sample cup.
5. Using a sterile pipette, obtain 3 mL of thaw/wash solution from the sterile sample cup; add to the resealable plastic bag containing the CBU contents that leaked when thawing.
6. Using a sterile pipette, remove the CBU and thaw/wash solution from the resealable plastic bag and place in a sterile sample cup.
7. Repeat steps 5 through 6 until all remaining CBU is transferred into a sample cup.
8. Using a sterile 20-mL syringe, draw the CBU and thaw/wash solution from the sterile sampling cups into the syringe; add to the Thaw/Wash Solution transfer pack. Repeat until all of the CBU and thaw/wash solution is transferred.

9. Using a sterile 20-mL syringe, remove the remaining thaw/wash solution from the Thaw/Wash Solution transfer pack; add to the CBU and Thaw/Wash Solution transfer pack.
10. When all of the thaw/wash solution in the Thaw/Wash Solution transfer pack has been transferred into the CBU and Thaw/Wash Solution transfer pack, mix the CBU and Thaw/Wash Solution transfer pack well by inverting 180° about 10 to 15 times.
11. Proceed to Section **D. Wash the Thawed CBU.**