

**Procedure Title: Cryopreservation of Hematopoietic Stem Cell Components**

**Procedure #: HSC.C101.01**

1. Reagents, Supplies, Equipment

Leibovitz's medium (L-15)  
Dimethyl sulfoxide (DMSO)  
Human serum albumin (HSA)  
Hetastarch (HES)  
Liquid nitrogen  
Labtop vial cooler  
Kyorack cold platform  
Cryocyte freezing bag (capacity 250ml)  
14 G needle  
60 ml sterile syringe  
2 ml cryovials  
15ml capped centrifuge tube  
Sampling site couplers  
Povidine iodine wipes  
Alcohol wipes  
Sebra tube sealer  
Plasma expressor  
Cryomed controlled rate freezer  
Floor centrifuge  
Refrigerator

2. Procedure

A. General

1. The final cell concentration in cryopreservative solution should not exceed:

For bone marrow (either purged or non- purged )  
or purged peripheral blood stem cells:  $40 \times 10^6$  cells/ml

For non- purged peripheral blood stem cells:  $200 \times 10^6$  cells/ml

2. Cool the cryopreservation solution at  $2^{\circ}$  to  $8^{\circ}$ C for at least 20 minutes

before adding to the cells.

3. Label cryocyte freezing bags, canisters, and vials. Refer to procedure HSC.E301.
4. For unpurged PBSCs, steps B and C are not necessary. Proceed with step D of this procedure. Refer to procedure HSC E301.

**B Prepare the initial resuspension and final cryopreservation solutions (for bone marrow procedures and purged PBSC only)**

Add the following relative quantities of reagent to a viaflex bag or sterile transfer pack of appropriate size in a laminar flow hood:

a. Bag1- Initial resuspension media  
L15 with HSA to 10% v/v

b. Bag2- Cryopreservation solution  
DMSO to 20% v/v  
HSA to 10% v/v  
HES to 3.0% v/v  
L-15 to final volume

**C. Wash out gentamicin (for bone marrow procedures and purged PBSC only)**

1. Clamp an aluminum ring securely to the exit tubing near the port of the cell suspension bag with hemostats. Tape the exit tubing in a coil.
2. Centrifuge the unit(s) at 400 x g at room temperature for 10 minutes  
Note: Be sure to secure exit tubing to the bag so that the spike is maintained in the upright position.
3. At the completion of centrifugation, place the unit onto the plasma expressor in the laminar flow hood. Position a sterile waste bottle to collect the supernatant.
4. Remove the aluminum clamp from the tubing with hemostats to express media to waste.
5. Express media to waste. Repeat for multiple cell collection bags. Disperse the cell pellet by using the side of the hand to smooth the bag. Use the “wash out gentamicin media” to combine multiple suspension bags into

one bag and to rinse each collection bag.

6. Fill the cell suspension bag with the remaining “wash out gentamicin” media. Repeat centrifugation and media expression (Steps C. 2. - 5. above) to complete the removal of gentamicin..

D. Add cryopreservation solution to the cells

1. Attach a needle to a sterile syringe. Place the bag containing the cells to be re-suspended on a kryorack that has just been removed from the freezer. Measure the pellet volume using the syringe and needle or by weight (volume in milliliters = weight in grams).
2. Using the syringe and needle to enter the bag containing L15 and HSA to 10% v/v. Add the media to the cells to (½) one half the final volume. Use the same syringe and slowly add an equal volume of ice cold cryopreservative solution to the cells on the Kryorack cold-pak surface.

E. Remove samples for testing and vial storage

1. Attach a sterile syringe to a needle.
2. Mix the cell suspension well. Remove the total volume of sample needed.
3. Aliquot samples. The following samples are required for any process:
  - a. Bacterial and fungal sterility to a sterile 15ml capped centrifuge tube (1ml).
  - b. White blood cell count (at least 0.1ml)
  - c. for all other future quality control testing (1ml per vial).
    - 6 cryovials for non purged PBSC's
    - 10 vials for non purged bone marrow
    - 10 vials for purged bone marrow or purged PBSC's
    - 3 vials for non purged bone marrow or PBSC's if purged marrow or PBSC have been frozen for infusion.

Note: Cryovials must be kept in a labtop vial cooler until placed into the controlled rate freezer.

- F. Dispense cryopreserved cell suspension to Fenwal cryocyte freezing bags in pre determined volumes.

- G. Seal the entry tubing using the Sebra Sealer (just above the bag) 3 times, overlapping each seal, and cut the tubing at the middle seal. Place the bags in (Pacific Science) aluminum freezing canisters with a pressure plate appropriate to the bag volume.

Note: For Cryocyte freezing bag volumes up to 70ml, use the large width pressure plate. For volumes greater than 70ml, use the smaller width pressure plate.

- H. Start the Cryomed programmable freezer. Initiate the program for freezing stem cells in cryocyte freezing bags. Begin the pre-cool phase of the program to maintain refrigerated temperature in the chamber. Refer to the Cryomed programmable freezer procedure HSC.D331. This step can be initiated at an appropriate time to allow the chamber temperature to equilibrate before the next step.
- I. Perform quality control checks to confirm that liquid nitrogen tanks are adequate and that the controlled rate freezer is adequately prepared for use. Document by recording a checkmark for each of the following system checks on the form “Controlled Rate Freeze System Checks”:
1. Check the liquid nitrogen supply tank level and pressure. The supply tank must at least be one half full. Read the level gauge on the tank. In addition, always evaluate the tank level by physical tilting to augment the level of assurance provided by the sometimes faulty gauge reading.
  2. Check the pen calibration of the controlled rate freezer chart recorder. Confirm that the chart recorder has an adequate paper supply to record the entire freeze cycle.
  3. During the freezing cycle, ensure early in the freeze run that the liquid nitrogen pressure in the supply tank is maintaining at least 10psi. Tanks that have been overfilled may discharge after appearing to be adequate upon initial inspection.
  4. Monitor the freezing curve closely through the SUPERCOOL phase of the program.
- J. Place the bags into the pre-cooled Cryomed freezer. Refer to the HSC.D331. Attach a flat temperature probe to a cryocyte freezing bag containing an appropriate volume of L15/HSA and cryopreservative solution. Place this bag into an aluminum canister. Place the bag and probe into the pre-cooled chamber.

- K. Remain in the wait phase of the program (pre- cool phase) until the sample temperature is within 2<sup>o</sup>C of the chamber temperature. Start the freezing program by pressing RUN.
  
- L. When the program is finished, remove the frozen stem cell units to a liquid nitrogen vapor storage tank until needed.