SOP: **Cryopreservation of hematopoietic cells from human leukapheresis product**
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Summary

Hematopoietic cells were provided as a service by the S. Heimfeld Laboratory at the Fred Hutchinson Cancer Research Center, Seattle, WA and include CD3+, CD4+, CD8+, CD14+, CD19+/CD20+, CD34+, and CD56+ cells, from both mobilized and non-mobilized donors. Cells were obtained from human leukapheresis product using standard procedures. Briefly, the lymphocyte subclasses were isolated by immunomagnetic separation using the CliniMACS affinity-based technology (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) according to the manufacturer's recommendations. Reagents, tubing sets, and buffers are purchased from Miltenyi Biotec.

The following protocol describes the cryopreservation of hematopoietic cells from human leukapheresis product.

Materials for Cryopreservation of Hematopoietic Cells

1. Normosol-R (Hospira, Inc., Lake Forest, IL, NDC# 0409-7967-03)
2. Human serum albumin (HSA) (CSL Behring, LLC, Kankakee, IL)
3. DMSO (Cryoserv cryopreservation solution, Edwards Life Sciences, Ben Venue Laboratories, Inc., Bedford, OH)
4. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
5. Corning conical centrifuge tubes (15mL and 50mL)
6. Graduated pipets (1, 5, 10, 25, 50mL)
7. Cryovials (Nunc, Cat# 368632)
8. Controlled-rate freezer (CryoMed Model #1010)
9. Hemocytometer
10. Thermolyne Locator 4 Liquid Nitrogen Freezer

This entire procedure should be done under sterile conditions.

1. Determine cell concentration of the cells to be frozen down per cryovial considering that an equal volume of cells is combined with an equal volume of freeze solution. For example, a total of $2x10^7$ cells to be divided into 1mL aliquots of $5x10^6$ cells for each cryovial, the cell volume needs to be 2mL and 2mL of freeze solution would be required.
2. Prepare an adequate volume of freeze solution for two-fold dilution of cells, based on the volume calculated in step# 1.
3. Freeze solution is composed of 40% chilled Normosol-R, 40% chilled HSA (human serum albumin), and 20% DMSO.
4. Begin preparation of the freeze solution by adding together the Normosol-R and HSA then slowly add the DMSO to the other ingredients while gently mixing. If a white precipitate occurs, then the DMSO was added too quickly.
5. Chill the freeze solution at 4°C for 10-15 minutes.

6. While the freeze solution is being prepared the cells can be brought to the correct volume based on the number of cryovials to be frozen down together with the cell concentration per vial.
7. Dispense cell suspension into cryovials, remembering to keep the cell suspension well mixed (usually 0.5mL cell suspension per vial).
8. Dispense an equal volume of chilled freeze solution to each cryovial (usually 0.5mL freeze solution per vial resulting in 1mL total volume per cryovial).
9. Cap tightly and gently mix right after the addition of the freeze solution.
10. Place the vials in a controlled rate freezer and use the "vial 1.1" program for freezing the vials. The freezer probe will be placed in a "mock" vial containing just PBS and freeze solution in it. The freeze program will decrease the temperature of the cell suspension approximately 1°C/minute. This process takes approximately 1 hour. At -80°C the program will be completed.
11. Once the freezing program is done, immediately place the vials into a liquid nitrogen freezer for storage.