SOP: **Slow thawing of cryopreserved hematopoietic cells from human leukapheresis product**
Date modified: **02/07/2011**
Modified by: **R. S. Hansen, T. K. Canfield (UW Stam Lab)**

Original Protocol supplied by: S. Heimfeld Laboratory, Fred Hutchinson Cancer Research Center, Seattle, WA

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. Cryopreservation of hematopoietic cells was performed according to the protocol entitled "SOP: Cryopreservation of hematopoietic cells from human leukapheresis product 02/07/2011 (UW Stam Lab)."

The following protocol describes the slow thawing of cryopreserved hematopoietic cells from human leukapheresis product.

Materials for Slow Thawing Cryopreserved Hematopoietic Cells

1. Phosphate Buffered Saline (1X PBS) (Cellgro, Cat# 21-040-CM)
2. Characterized Fetal Bovine Serum (FBS) (HyClone, Cat# SH30071)
3. 70% Ethanol
4. 0.22µm Corning Filter Systems (Cat# 431097 for 500mL and Cat# 431098 for 1L)
5. Corning conical centrifuge tubes (15mL and 50mL)
6. Graduated pipets (1, 5, 10, 25, 50mL)
7. Hemocytometer
8. Eppendorf Centrifuge 5810R
9. Thermolyne Locator 4 liquid nitrogen freezer
10. 37°C water bath

Cell Slow Thawing Procedure

1. Remove cells from liquid nitrogen storage and thaw rapidly in a 37°C water bath.
2. Swab outside surface of cryovial with 70% ethanol and transfer cells to 50mL conical centrifuge tube.
3. Dilute cells with cell thawing buffer (0.22µm filter-sterilized room temperature PBS supplemented with 1% FBS) by making four dilutions as follows (from a starting cell volume of 1mL):
    a. add 1mL thawing buffer slowly, dropwise, mixing with slow, gentle swirling and let equilibrate for 3 min (2mL total volume).

   b. add 2mL thawing buffer slowly, dropwise, mixing with slow, gentle swirling and let equilibrate for 3 min (4mL total volume).
   c. add 8mL thawing buffer slowly, mixing with slow, gentle swirling and let equilibrate for 3 min (12mL total volume).
   d. add 20mL thawing buffer slowly, mixing with slow, gentle swirling and let equilibrate for 3 min (32mL final total volume).
4. Centrifuge at 470 x g for 10 min at room temperature.
5. Carefully remove supernatant and disturb pellet by raking the tube bottom against a tube rack.
6. Wash once with thawing buffer as in steps 4 and 5, and resuspend in cold 1X PBS for further processing. Do a cell count with the hemocytometer and determine the amount of cells necessary for DNaseI treatment, crosslinking for ChIP, and/or RNA experimentation.
7. Proceed with the protocol entitled “SOP: Human hematopoietic cells: DNaseI treatment, crosslinking, and preserving cells for RNA 02/07/2011 (UW Stam Lab).”