SOP: **Isolation of CD34-positive cells from human leukapheresis product**
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Summary

CD34-positive cells (CD34+) were provided as a service by the S. Heimfeld Laboratory at the Fred Hutchinson Cancer Research Center. The cells were obtained from human leukapheresis product derived from mobilized donors using standard procedures. Briefly, donors were treated with human granulocyte colony-stimulating factor (G-CSF), blood cells were collected by apheresis, and CD34+ cells were isolated by immunomagnetic separation using the CliniMACS affinity-based technology (CliniMACS CD34 MicroBeads; Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) according to the manufacturer's recommendation. Reagents, tubing sets, and buffers are purchased from Miltenyi Biotec. The cells are provided either as freshly isolated or as cryopreserved and these were then processed further (e.g., for ChIP, RNA, DNA, and/or DNaseI).

The CD34 antigen is a single transmembrane glycoprotein that is mainly expressed on human hematopoietic stem and progenitor cells but is also present on endothelial progenitor cells. The CD34 antigen is involved in cell adhesion and is thought to function as a signaling molecule.

Materials for Thawing Cryopreserved CD34+ Cells (if applicable)

1. Thermolyne Locator 4 liquid nitrogen freezer
2. 70% Ethanol
3. Characterized Fetal Bovine Serum (HyClone, Cat# SH30071)
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. Eppendorf Centrifuge 5810R

CD34+ Cell Thawing Procedure

1. Remove cells from liquid nitrogen storage and thaw rapidly in a 37°C water bath.
2. Swab outside surface of cryotube with 70% ethanol and transfer cells to 50mL conical tube.
3. Dilute cells with cell thawing buffer (PBS with 1% FBS warmed to room temperature) by making four dilutions as follows (for a starting cell volume of 1mL):
    a. add 1mL thawing buffer with slow, gentle mixing and let equilibrate for 3 min (2mL total).
    b. add 2mL thawing buffer with slow, gentle mixing and let equilibrate for 3 min (4mL total).
    c. add 8mL thawing buffer with slow, gentle mixing and let equilibrate for 3 min (12mL total).
    d. add 20mL thawing buffer with slow, gentle mixing and let equilibrate for 3 min (32mL total).
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 the desired buffer and volume for further processing.