SOP: **Isolation of CD14-positive cells from human leukapheresis product**
Date modified: **1/3/2011**
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Summary

CD14+ cells 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 using standard procedures. Briefly, CD14+ cells 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 cells are provided either as freshly isolated or as cryopreserved and these were then processed further (e.g., for ChIP, RNA, DNA, or DNaseI).

The CD14 antigen is a high-affinity receptor for the lipopolysaccharide (LPS) and LPS-binding protein (LPB) complex. CD14 is strongly expressed on the surface of most human monocytes and macrophages.

Materials for Thawing Cryopreserved CD14+ 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

CD14+ 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.