SOP: **Propagation of Th2**
Date modified: **4/20/2009**
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Source Information:

Cells are procured from primary pheresis of a single normal subject.

Notes:

Th2 T-cell subset is purified and expanded in primary culture.

Materials List

1. Naïve CD4+ T cell isolation kit (Miltenyi Biotech, Cat # 130-091-894)
2. autoMACS Separator (Miltenyi Biotech)
3. Aim V medium (Invitrogen, Cat # 087-0112DK)
4. AB serum (Lonza Bioscience, Cat # 14-490E)
5. Anti-CD3/Anti-CD28 coated beads (Dynal/Invitrogen, Cat #111-31D)
6. Human IL-14 (R&D Systems)
7. Anti-Human IL-12 (eBioscience)
8. Anti-Human INFg (eBioscience)
9. Bio-Plex Human Cytokine Th1/Th2 Panel (Bio-Rad, Cat # 171-A11081)
10. T25 & T225 culture flasks
11. Graduated pipets
12. Hemocytometer
13. Phorbol 12-myristate 13-acetate (Sigma, Cat # P1585)
14. Ionomycin (Sigma, Cat # I3909)

Th2 Polarization Medium

Aim V medium
2% AB serum
Human IL-14 (10 ng/mL)
Anti-Human IL-12 (5 ug/mL)
Anti-Human INFg (5 ug/mL)

Procedure

A. Isolation of naïve CD4+ T cells

1) Isolate naïve CD4+ T cells by negative selection using the Naïve CD4+ T Cell Isolation Kit according to manufacturer's recommendations.

B. Stimulation and polarization of cells

1) Resuspend naïve CD4+ T cells (93% $\geq$ CD4+CD45RA+) in Aim V medium containing 2% serum.
2) Stimulate cells with anti-CD3 and anti-CD28 coated beads in polarizing medium.
3) Expand cells in culture for 7-10 days.
4) If needed, stimulate cells with PMA (2.5 ug/mL) and Ionomycin (500 uM) to divide.

C. Confirmation of Th1 polarization

1) Assay supernatant with Bio-Plex Human Cytokine Th1/Th2 panel according to manufacturer's protocol.

D. Harvest

1) Pellet cells by centrifugation and wash cells in 1X PBS.
2) Examine viability using trypan blue staining.

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