Primary Cord Naïve CD4+ CD45RA+ Cells

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Goal: To obtain large numbers of highly pure primary CD4+ CD45RO- CD25- cells from cord blood, and prepare them as naïve, Th0, and Th1 cells. This protocol combines two purification strategies, a negative selection step with Rosette-Sep Reagents followed by an additional negative selection step with magnetic goat anti-mouse beads, to give desired purity but with higher yields than can be obtained with FACS sorting and cells are "untouched" with no antibodies bound to their surface.

Reagents:

1) Rosette-sep CD4 reagent
2) Lympocyte separation media
3) Mouse anti-human CD45RO, mouse anti-human CD25, and mouse anti-human CD161 antibodies (eBioscience or BD Bioscience)
4) Goat anti-mouse magnetic particles (Chemicell)
5) Miltenyi LS magnetic separation columns
6) PBE buffer (PBS + 0.5%BSA + 2 mM EDTA)
7) T Cell Media = RPMI 1640 + 10%FCS + Pen/Strep + 2mM L-glut + 10mM Hepes + 50μM 2-βME
8) 2X Freezing media = 8ml FCS + 2ml DMSO
9) Human recombinant IL-12 (R&D Systems)

Step 1: Negative Selection of CD4 Cells with Rosette Sep

1) Obtain sample of cord blood anti-coagulated with 10u/ml heparin
2) Dilute 1:2 with PBE
3) Underlay with lymphocyte separation media
4) Spin at 1200g x 30 minutes at RT. Collect CD4 T cells at interface.
5) Wash in PBE and count.

Step 2: Further enrichment with Gt anti-mouse beads

1) Add 0.05ug of anti-CD25, anti-CD161, and anti-CD45RO per million cells
2) Incubate on ice 30 minutes
3) Wash once with PBE
4) Add 3ul per million of goat anti-mouse magnetic particles.
5) Incubate on ice 30 minutes.
6) Wash once with PBE.
7) Resuspend in PBE, then run through Miltenyi LS column on magnet.
8) Wash column 3 times with PBE, collecting the flow-through.
9) Count cells, spin down, and resuspend in T Cell Media.

Step 3: Stimulation for DNAse I experiments

1) For Naïve Cells, take 10 million in T Cell Media. Add 2X Freezing media and freeze immediately.

2) For Naïve Cells with Short Stim, Take 10 million and stimulate in T cell media at 37° and 7% CO_2 with plate-bound anti-CD3 + anti-CD28 for 4 hours. Then freeze cells.
3) For Th0 Cells, take 10 million and culture with CD3/CD28 beads with no cytokines added (neutral conditions) in T cell media at 37° and 7% CO_2 for 3 days. Remove beads, collect cells, and freeze.
4) For Th1 Cells, take 10 million and culture with CD3/CD28 beads + 1 ng/ml IL-12 (Th1 conditions) in T cell media at 37° degrees and 7% CO_2 for 3 days. Remove beads, collect cells, and freeze.