Macs-based enrichment of untouched CD4+CD8+ thymocytes for biochemistry

CD53 depletion protocol

1) Dissect thymuses into Medium-199 (+ 2% FCS, 1x Pen/Strep, 1X Hepes)
2) Make single cell suspensions
3) Stain thymocytes in 0.5 – 1 ml MACS running buffer (DPBS, 0.5% BSA, 2mM EDTA)
   with anti-CD53 (Pharmingen cat # 559364 for 15 min on ice
   50ul per 2E8 cells, 75ul for TCR transgenics)
4) Wash with 10-20X volume of MACS running buffer.
5) Aspirate buffer completely (important!)
6) Stain thymocytes in 0.5 – 1 ml MACS running buffer 15 min on ice
   with anti-rat IgG-biotin (Jackson Immunoresearch cat # 112-065-143
   100ul per 2E8 cells, 150ul for TCR transgenics)

   OR: with anti-rat IgM-biotin
   20 ul per 2E8 cells
7) Wash with 10-20X volume of MACS running buffer.
8) Add Miltenyi Biotech anti-biotin beads and stain on ice 15 min
   (100ul per 2E8 cells), with intermittent mixing.
9) Wash with 10-20X volume of MACS running buffer.
10) Resuspend in 2 ml MACS running buffer and separate on MACS column.
11) Assess purity by flow cytometry.