Protocol 209_ Cryopreservation of primary $CD4^+$ T cells

Reagents:

- IMDM 10% FCS
- 0.83% NH_4Cl in water (sterile filtered)
- Freezing medium: 90% DMSO, 10% FCS
- FCS
- FACS antibodies of your choice
- Cryo vials
- For CD4 MACS (protocol 301: Positive cell enrichment by MACS)
    - MACS pre-separation filters (Miltenyi, # 130-041-407)
    - MS columns (Miltenyi, #130-042-201)
    - OctoMACS™ Separation Unit (Miltenyi, # 130-042-109)
    - MACS buffer: 2% FCS (20 ml), 2 mM EDTA (4 ml of 0.5 M EDTA stock), 976 ml 1×PBS. Filter sterilize. Store at 4˚C. Keep sterile!
    - CD4 microbeads

Method:
All steps are done sterilely!

1. Homogenize spleen into single cell suspension in IMDM 10% FCS
2. Centrifuge for 5 min at 1500 rpm
3. Lyse red blood cells: Resuspend 1 spleen pellet into 1 ml 0.83% NH_4Cl red cell lysis buffer
4. Incubate for <u>exactly</u> 5 min at RT
5. Fill up with IMDM 10% FCS
6. Centrifuge for 5 min at 1500 rpm
7. Enrich for $CD4^+$ T cells according to protocol 301
8. Stain with antibodies for FACS sort <u>in MACS buffer</u>
   <u>E.g:</u> naïve $CD4^+$ T cells are $CD4^+CD25^-CD45RB^{high}$: CD25 PerCPCy5.5 (#3, IgG1 λ), CD4 APC (#1, IgG2a, κ), CD45RB Pacific Blue (#9, IgG2s, κ)
9. Prepare 15 ml Falcon tubes containing 2 ml FCS. These will be used to collect the sorted cells
10. After cells are sorted, centrifuge for 5 min at 1500 rpm
11. Resuspend in freezing medium at $1\text{-}2\times10^6$/ml
12. Aliquot 1 ml cell suspension per cryo vial
13. Cryopreserve using the controlled rate freezer (CMF program to -30°C) and transfer directly to liquid nitrogen

Notes:

About 50% of the cells will be dead after thawing

Use the dead cell removal kit (#130-090-101, Miltenyi) to remove dead cells prior to use