Protocol 301_Positive cell enrichment by MACS

Reagents:

- 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!
- Microbeads of your choice (CD19, CD4, CD11c…)

Method:

1. Homogenize organs into single cell suspensions
2. Transfer obtained single cell suspension into a 15 ml conical tube. By doing this, the amount of cells lost after centrifugation will be minimized.
3. Centrifuge for 5 min at 1300 rpm
4. Resuspend cell suspension (up to 2×10^8 unlabeled cells and up to 10^7 magnetically labeled cells) in 300 μl MACS buffer and 40 μl MACS antibody
5. Incubate for 30 min at 4°C and flick tube from time to time
6. Wash with MACS buffer and centrifuge for 5 min at 1300 rpm
7. Resuspend in 500 μl MACS buffer
8. Mount MACS pre-separation filters on top of MS columns and pre-wet with 500 μl MACS buffer
9. Apply cell suspension through MACS pre-separation filter-MS column
10. Wash column 3× with 500 μl MACS buffer, adding buffer each time the column reservoir is empty
11. Flush out magnetically labeled cell fraction with 1 ml MACS buffer by firmly applying the supplied plunger with the column
12. Centrifuge for 5 min at 1300 rpm

Notes:

- For B cell sorting (with CD19 micobeads), Peyer's patches are NOT sorted because the majority of the cells are B cells
- It is recommended to test via flow cytometry the unsorted, positive and negative fractions with the markers of inerest. In this way, the purity of the sorted fraction can be determined.
- For a positive sort, the magnetically labelled fraction is collected.
- For a negative sort, the flow through is collected.