Protocol 208_DC isolation from lymph node

- *Digestion medium*: RPMI 2% FBS 1mg/ml collagenase et 0.1 mg/ml DNase
    - RPMI 1640 (GIMCO-ref 31870-025)
    - FBS- FŒTAL BOVINE SERUM (GIMCO ref 10270-106)
    - COLLAGENASE TYPE IA (SIGMA C9891-1G)
    - DNase I (ROCHE 11 284 932 001)
- *Stop medium*: RPMI 2% FBS 2mM EDTA
    - EDTA 0.5M ph 8.0 (GIMCO 15575-038)
- *Complete medium*: RPMI 10% FBS 10mM HEPES 0.05 mM 2-mercaptoethanol 2mM Glutamine, 1% Penicillin-Streptomycin.
    - HEPES 1M (GIMCO 15140-122)
    - 2-mercaptoethanol 50mM (GIMCO 31350-010)
    - Glutamine 200mM (Invitrogen 25030123)
    - Penicillin-Streptomycin (GIMCO 15140-122)

1. Isolate lymph nodes and put in RPMI into a 2 mL eppendorf. Keep on ice.
2. Eliminate RPMI medium and tear up ganglions with scissors directly in the eppendorf.
3. Add 2 ml digestion medium into a 2 mL eppendorf.
4. Incubation 20-25 min at 37°C in the incubator with 240 rpm shaking
5. Flush and filter in 50 mL tube and wash the cells by adding buffer to a final volume of 20 ml.
6. Spin 1300 rpm for 7 min, 4 ºC
7. Count the cells.

a. Purifier DC

- *Macs medium:* PBS 0.5% BSA 2mM EDTA
    - DPBS 10X –CaCl2 – MgCl2 (GIMCO 14200-059)
    - BSA- albumin from bovine serum 98% (SIGMA A7906)
- CD11c microBeads mouse
    - Clone N418 (MACS miltenyibiotec 130-052-001)

1. Centrifuge cell suspension at 1300 rpm for 7 min, 4 ºC. Pipette off supernatant completely.
2. Resuspend cell pellet in 250 10^6 cell/ml of macs medium
3. Add 1µl of CD11c (N418) MicroBeads par 10^6 total cells.
4. Mix well and incubate for 20 minutes at 4 ºC
5. Wash the cells by adding macs medium to final volume and centrifuge.
6. Resuspend up at 3 ml of macs medium.

- Choose an appropriate MACS column (LS 100 10^6 positive cell for 2 10^9 total cells)
- Place column in the magnetic field of a suitable MACS Separator
- Rinsing column with 3 ml of Macs medium
- Apply cell suspension onto the column
- Collect unlabeled cells and wash column with 2x3 ml of Macs medium
- Remove column from the separator and place it on a suitable collection tube
- Add 5 ml complete medium onto the column. Immediately flush out labelled cells by firmly applying plunger supplied with the column.
- Centrifuge and count the cells.