Th2 Polarization of Mouse CD4+ Cells V.3

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Materials

MATERIALS

MojoSort™ Mouse CD4 T Cell Isolation Kit **BioLegend Catalog #**480005, 480006, 480033

RBC Lysis Buffer **BioLegend Catalog #**420301

Anti-mouse CD3ε clone 145-2C11 (Ultra-LEAF™ format) **BioLegend Catalog #**100339

Anti-mouse CD28 clone 37.51 (Ultra-LEAF™ format) **BioLegend Catalog #**102116

Monensin Solution **BioLegend Catalog #**420701

Recombinant mouse IL-2 (carrier-free) **BioLegend Catalog #**575402

Recombinant mouse IL-4 (carrier-free) **BioLegend Catalog #**574302

- Sterile PBS
- Cell culture medium (RPMI 1640 supplemented with 10% FBS)
- Sterile plastic petri dishes
- Sterile T-75 culture flask
- Concanavalin A (Con A) (Sigma, Cat. No. C5275)

Isolation of CD4+ Cells From Lymph Nodes

1 Harvest lymph nodes (superficial cervical, mandibular, axillary, inguinal, and mesenteric) from mice.

2 Tease lymph nodes through a sterile 70-µm nylon cell strainer to obtain single-cell suspensions in complete RPMI containing 10% FCS (complete medium).

3 Resuspend cells in complete medium and use your favorite method to isolate $CD4^+$cells. Consider using our **Mojosort™ Mouse CD4 T Cell Isolation Kit.**

Th2 Polarization of CD4+ Cells

4 On day 0, plate $CD4^+$cells at 30 $\times 10^6$/30 ml/T-75 flask. Culture cells for 3 days in complete RPMI containing 10% FCS, Con A (5µg/mL), recombinant mouse IL-2 (20ng/ml), and recombinant mouse IL-4 (50ng/ml).

5 On day 3, harvest the cells and wash cells once. Seed 15 $\times 10^6$cells/30 ml/T-75 flask along with recombinant mouse IL-2 (20ng/ml) and recombinant mouse IL-4 (50ng/ml).

6 On day 5, coat a 60 × 15 mm tissue culture petri dish with anti-mouse CD3ε, clone 145-2C11, 10µg/mL in PBS, 5ml/dish. Incubate in a 4°C refrigerator overnight.

7 On day 6, wash the anti-mouse CD3ε pre-coated tissue culture petri dish with PBS. Harvest the cells from the flask (that were seeded on Day 5), wash them twice, and seed at 20 $\times 10^6$/10 ml/petri dish along with 10µl of monensin (1000x) and anti-mouse CD28, clone 37.51 (5µg/ml). Incubate for 6 hours at 37°C in a CO^2 incubator.

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8 After harvesting, the cells are ready for staining.