



Th2 Polarization of Mouse CD4+ Cells V.2

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Materials

- Sterile PBS
- Cell culture medium (RPMI 1640 supplemented with 10% FBS)
- Sterile plastic petri dishes
- Sterile T-75 culture flask
- RBC Lysis Buffer (Cat. No. [420301](#))
- Concanavalin A (Con A) (Sigma, Cat. No. C5275)
- Anti-mouse CD3 ϵ , clone 145-2C11 (LEAF[™] format, Cat. No. [100314](#))
- Anti-mouse CD28, clone 37.51, (LEAF[™] format, Cat. No. [102112](#))
- Recombinant mouse IL-2 (carrier-free) (Cat. No. [575402](#))
- Recombinant mouse IL-4 (carrier-free) (Cat. No. [574302](#))
- Monensin Solution (Cat. No. [420701](#))
- Mouse MojoSort[™] CD4 T-cell Isolation Kit (Cat. No. [480005](#))



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- μ m 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 30×10^6 /30 ml/T-75 flask. Culture cells for 3 days in complete RPMI containing 10% FCS, Con A (5 μ g/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 15×10^6 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 $\times$ 15 mm tissue culture petri dish with anti-mouse CD3 ϵ , clone 145-2C11, 10 μ g/mL in PBS, 5ml/dish. Incubate in a 4°C refrigerator overnight.
- 7 On day 6, wash the anti-mouse CD3 ϵ 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 20×10^6 /10 ml/petri dish along with 10 μ l of monensin (1000x) and anti-mouse CD28, clone 37.51 (5 μ g/ml). Incubate for 6 hours at 37°C in a CO₂ incubator.

 06:00:00
- 8 After harvesting, the cells are ready for staining.