Th2 Polarization of Mouse CD4+ Cells V.3

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MATERIALS

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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)

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External link:

<https://www.biolegend.com/protocols/th2-polarization-of-mouse-cd4--cells-protocol/4238/>

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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 x 10⁶/30 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 x 10⁶ 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 x 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 x 10⁶/10 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 CO₂ incubator.

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