Th2 Polarization of Mouse CD4+ Cells

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[1]BioLegend

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Version 2

Working

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EXTERNAL LINK

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

PROTOCOL STATUS

Working

MATERIALS TEXT

- 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 CD3ε, 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-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 $x10^6$/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 $x10^6$cells/30 ml/T-75 flask along with recombinant mouse IL-2 (20ng/ml) and recombinant mouse IL-4 (50ng/ml).

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

6 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 x10^6/10 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^2incubator.

06:00:00

8 After harvesting, the cells are ready for staining.