



Th2 Polarization of Mouse CD4+ Cells V.3

DOI

dx.doi.org/10.17504/protocols.io.796hr9e



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

Protocol Citation: Sam Li . Th2 Polarization of Mouse CD4+ Cells. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.796hr9e>

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Created: October 16, 2019



Last Modified: October 16, 2019

Protocol Integer ID: 28702

Keywords: th2 polarization of mouse cd4, polarization of mouse cd4, th2 polarization, mouse cd4, cell, polarization, mouse

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)

Troubleshooting




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.