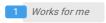




Th2 Polarization of Mouse CD4+ Cells V.3 👄

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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/

## MATERIALS

NAME Y	CATALOG # \	VENDOR V
MojoSort™ Mouse CD4 T Cell Isolation Kit	480005, 480006, 480033	BioLegend
RBC Lysis Buffer	420301	BioLegend
Anti-mouse CD3ε clone 145-2C11 (Ultra-LEAF™ format)	100339	BioLegend
Anti-mouse CD28 clone 37.51 (Ultra-LEAF™ format)	102116	BioLegend
Monensin Solution	420701	BioLegend
Recombinant mouse IL-2 (carrier-free)	575402	BioLegend
Recombinant mouse IL-4 (carrier-free)	574302	BioLegend

## MATERIALS TEXT

- 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.
- Tease lymph nodes through a sterile 70-μm nylon cell strainer to obtain single-cell suspensions in complete RPMI containing 10% FCS (complete medium).
- Resuspend cells in complete medium and use your favorite method to isolate CD4<sup>+</sup>cells. Consider using our <u>Mojosort™ Mouse</u>
  <u>CD4 T Cell Isolation Kit.</u>

## Th2 Polarization of CD4+ Cells

- 4 On day 0, plate CD4<sup>+</sup>cells at 30 x10<sup>6</sup>/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).
- On day 3, harvest the cells and wash cells once. Seed 15 x10<sup>6</sup> 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 x10<sup>6</sup>/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<sup>2</sup> incubator.

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8 After harvesting, the cells are ready for staining.

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