



Oct 16, 2019

Th2 Polarization of Mouse CD4+ Cells V.3 [↗](#)Sam Li<sup>1</sup><sup>1</sup>BioLegend

1

Works for me

[dx.doi.org/10.17504/protocols.io.796hr9e](https://doi.org/10.17504/protocols.io.796hr9e)

BioLegend

Sam Li  
BioLegend

## EXTERNAL LINK

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

## MATERIALS

NAME <a href="#">▼</a>	CATALOG # <a href="#">▼</a>	VENDOR <a href="#">▼</a>
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.
- 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<sup>+</sup> cells. Consider using our [Mojosort™ Mouse CD4 T Cell Isolation Kit](#).

## Th2 Polarization of CD4<sup>+</sup> Cells

- 4 On day 0, plate CD4<sup>+</sup> cells at 30 x 10<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).
- 5 On day 3, harvest the cells and wash cells once. Seed 15 x 10<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 x 10<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<sub>2</sub> incubator.  
 06:00:00
- 8 After harvesting, the cells are ready for staining.



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited