



Th1 Polarization of Mouse CD4+ Cells [↗](#)

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¹BioLegend

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Version 2

BioLegend

Working



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EXTERNAL LINK

<https://www.biolegend.com/protocols/th1-polarization-of-mouse-cd4--cells-protocol/4242/>

PROTOCOL STATUS

Working

GUIDELINES

Reagent List:

- Sterile PBS
- Cell culture medium (RPMI 1640 supplemented with 10% FBS)
- Sterile 12-well plate
- Sterile 6-well plate
- RBC Lysis Buffer (Cat. No. 420301)
- Anti-mouse CD3ε, clone 145-2C11 (LEAF™ format, Cat. No. 100314)
- Anti-mouse CD28, clone 37.51, (LEAF™ format, Cat. No. 102112)
- Anti-mouse IL-4, clone 11B11, (LEAF™ format, Cat. No. 504108)
- Recombinant mouse IL-2 (carrier-free) (Cat. No. 575402)
- Recombinant mouse IL-12 (p70) (carrier-free) (Cat. No. 577002)
- Monensin Solution (Cat. No. 420701)
- PMA (Phorbol 12-myristate 13-acetate) (Cat. No. P8139 from Sigma)
- Ionomycin (Cat. No. I0634 from Sigma)

MATERIALS

	NAME	CATALOG #	
	RBC Lysis Buffer	420301	by BioLegend
	Anti-mouse CD3ε, clone 145-2C11 (LEAF™ format)	100314	by BioLegend
	Anti-mouse CD28, clone 37.51, (LEAF™ format)	102112	by BioLegend
	LEAF™ Purified anti-mouse IL-4 Antibody, clone 11B11	504108	by BioLegend
	Recombinant Mouse IL-2 (carrier-free)	575402	by BioLegend
	Recombinant Mouse IL-12 (p70) (carrier-free)	577002	by BioLegend
	Monensin Solution (1,000X)	420701	by BioLegend
	Phorbol 12-myristate 13-acetate (PMA)	P8139	by Sigma Aldrich
	Ionomycin calcium salt from Streptomyces conglobatus	I0634	by Sigma Aldrich

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 incomplete 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.

Th1 Polarization of CD4⁺ Cells


- 4 On day 0, coat 12-well plate with anti-mouse CD3ε, clone 145-2C11 (3 µg/ml). Incubate at 37°C for 2 hours or 4°C overnight. Aseptically decant antibody solution from the plate. Wash plate 3 times with sterile PBS. Discard liquid.

 02:00:00

- 5 Plate CD4⁺ cells at 1.0×10^6 /1ml/well. Culture cells for 5 days at 37°C, 5% CO₂, in the presence of anti-mouse CD28, clone 37.51 (3 µg/mL), anti-mouse IL-4, clone 11B11 (10 µg/mL), recombinant mouse IL-2 (5 ng/mL), and recombinant mouse IL-12 (10 ng/ml).

- 6 On day 3, if media is yellow, add 2 ml/well of fresh media.

- 7 On day 5, wash cells once and then restimulate in complete media with 50 ng/ml PMA, 1 µg/ml ionomycin and 10 µl monensin (1000x), in a 6-well plate in incubator at 37°C for 5 hours.

 05:00:00

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



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