



Oct 16, 2019

Th9 Polarization of Mouse CD4⁺ Cells V.3 [↗](#)Sam Li¹¹BioLegend

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Works for me

[dx.doi.org/10.17504/protocols.io.798hr9w](https://doi.org/10.17504/protocols.io.798hr9w)

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EXTERNAL LINK

<https://www.biolegend.com/protocols/th9-polarization-of-mouse-cd4--cells-protocol/4243/>

MATERIALS

NAME	CATALOG #	VENDOR
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
Anti-mouse IFN-γ clone XMG1.2 (Ultra-LEAF™ format)	505834	BioLegend
Recombinant human TGF-β1 (carrier-free)	580702	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 (IMDM supplemented with 10% FBS)
- Sterile plastic petri dishes
- PMA (Phorbol 12-myristate 13-acetate) (Cat. No. P8139 from Sigma)
- Ionomycin (Cat. No. I0634 from Sigma)

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 IMDM 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](#).

Th9 Polarization of CD4+ Cells:

- 4 On day 0, coat 60 x 15mm of plastic petri dishes with anti-mouse CD3 ϵ , clone 145-2C11 (5 μ g/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 10×10^6 /5 ml/dish. Culture cells for 3 days in the presence of anti-mouse CD28, clone 37.51 (5 μ g/mL), recombinant human TGF- β 1 (10ng/mL), recombinant mouse IL-4 (10ng/mL), recombinant mouse IL-2 (20ng/ml), and anti-mouse IFN- γ , clone XMG1.2 (10 μ g/mL).
- 6 On day 3, wash cells once and then restimulate in complete medium with 500ng/ml PMA and 500ng/mL ionomycin, in the presence of monensin for 6 hours.  06:00:00
- 7 After harvesting, the cells are ready for staining.

Note: Recombinant human TGF- β is effective for stimulating mouse cells.



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