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Th1 Polarization of Mouse CD4+ Cells V.3

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[1]BioLegend

1 Works for me dx.doi.org/10.17504/protocols.io.795hr86

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EXTERNAL LINK

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

MATERIALS

NAME	CATALOG #	VENDOR
Phorbol 12-myristate 13-acetate (PMA)	P8139	Sigma Aldrich
Ionomycin calcium salt from Streptomyces conglobatus	I0634	Sigma Aldrich
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 IL-4 clone 11B11 (Ultra-LEAF™ format)	504122	BioLegend
Monensin Solution	420701	BioLegend
Recombinant mouse IL-2 (carrier-free)	575402	BioLegend
Recombinant mouse IL-12 (p70) (carrier-free)	577002	BioLegend

MATERIALS TEXT

- Sterile PBS
- Cell culture medium (RPMI 1640 supplemented with 10% FBS)
- Sterile 12-well plate
- Sterile 6-well plate

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 (3µg/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 x 10^6 /1ml/well. Culture cells for 5 days at 37°C, 5% CO_2, 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.