Oct 15, 2019

Th17 Polarization of Mouse CD4+ Cells V.3

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[1]BioLegend

1 Works for me dx.doi.org/10.17504/protocols.io.79vhr66

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EXTERNAL LINK

https://www.biolegend.com/protocols/th17-polarization-of-mouse-cd4--cells/4284/

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
Anti-mouse IL-4 clone 11B11 (Ultra-LEAF™ format)	504122	BioLegend
Recombinant mouse IL-6 (carrier-free)	575704	BioLegend
Recombinant mouse IL-23 (carrier-free)	589002	BioLegend
Recombinant human TGF-β1 (carrier-free)	580702	BioLegend
Brefeldin A	420601	BioLegend
Monensin Solution	420701	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.

Th17 Polarization of CD4+ Cells

4 On day 0, coat 60 x 15mm of plastic petri dishes with anti-mouse CD3ε, clone 145-2C11 (5µg/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 x 10^6/5 ml/dish. Culture cells for 4 days in the presence of anti-mouse CD28, clone 37.51 (5µg/mL), recombinant mouse IL-6 (50ng/mL), recombinant human TGF-β1 (1ng/mL), recombinant mouse IL-23 (5ng/ml), anti-mouse IL-4 (10µg/mL), and anti-mouse IFN-γ (10µg/mL).

6 On day 3, slowly add 5ml of fresh media along with same the concentration of antibodies/cytokines as used on day 0.

7 On day 4, wash cells once and then restimulate in complete medium with 500ng/ml PMA and 500ng/mL ionomycin, in the presence of Brefeldin A (If you are looking for IL-21 production, use monensin) for 4-5 hours.

05:00:00

8 After harvesting, the cells are ready for staining.

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