Version 2

Working

Th17 Polarization of Mouse CD4+ Cells

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EXTERNAL LINK

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

PROTOCOL STATUS

Working

MATERIALS TEXT

- Sterile PBS
- Cell culture medium (IMDM supplemented with 10% FBS)
- Sterile plastic petri dishes
- 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 IFN-γ, clone XMG1.2, (LEAF™ format, Cat. No. 505812)
- Mouse MojoSort™ CD4 T-cell Isolation Kit (Cat. No. 480005)
- Anti-mouse IL-4, clone 11B11, (LEAF™ format, Cat. No. 504108)
- Recombinant mouse IL-6 (carrier-free) (Cat. No. 575704)
- Recombinant mouse IL-23 (carrier-free) (Cat. No. 589002)
- Recombinant human TGF-β1 (carrier-free) (Cat. No. 580702)
- Monensin Solution (Cat. No. 420701)
- PMA (Phorbol 12-myristate 13-acetate) (Cat. No. P8139 from Sigma)
- Ionomycin (Cat. No. I0634 from Sigma)
- Brefeldin A (Cat. No. 420601)

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 10×10^6/5 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.