



Th17 Polarization of Mouse CD4+ Cells V.2

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Materials

- Sterile PBS
- Cell culture medium (IMDM supplemented with 10% FBS)
- Sterile plastic petri dishes
- RBC Lysis Buffer (Cat. No. **420301**)
- Anti-mouse CD3 ϵ , 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- β 1 (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**)



Troubleshooting



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- μ m 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 × 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 × 10⁶/5 ml/dish. Culture cells for 4 days in the presence of anti-mouse CD28, clone 37.51 (5 μ g/mL), recombinant mouse IL-6 (50ng/mL), recombinant human TGF- β 1 (1ng/mL), recombinant mouse IL-23 (5ng/ml), anti-mouse IL-4 (10 μ g/mL), and anti-mouse IFN- γ (10 μ g/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.