



Th9 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**)
- Mouse MojoSort™ CD4 T-cell Isolation Kit (Cat. No. **480005**)
- Anti-mouse IFN- γ , clone XMG1.2, (LEAF™ format, Cat. No. **505812**)
- Recombinant mouse IL-2 (carrier-free) (Cat. No. **575402**)
- Recombinant mouse IL-4 (carrier-free) (Cat. No. **574302**)
- 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)



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**](#).

Th9 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 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.