



Th1 Polarization of Mouse CD4+ Cells V.2

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Keywords: polarization of mouse cd4, mouse cd4, polarization, cell, mouse

Guidelines

Reagent List:

- Sterile PBS
- Cell culture medium (RPMI 1640 supplemented with 10% FBS)
- Sterile 12-well plate
- Sterile 6-well plate
- 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 IL-4, clone 11B11, (LEAF™ format, Cat. No. 504108)
- Recombinant mouse IL-2 (carrier-free) (Cat. No. 575402)
- Recombinant mouse IL-12 (p70) (carrier-free) (Cat. No. 577002)
- Monensin Solution (Cat. No. 420701)
- PMA (Phorbol 12-myristate 13-acetate) (Cat. No. P8139 from Sigma)
- Ionomycin (Cat. No. I0634 from Sigma)

Materials

MATERIALS

⊗ RBC Lysis Buffer **BioLegend Catalog #420301**

⊗ Anti-mouse CD3 ϵ , clone 145-2C11 (LEAF™ format) **BioLegend Catalog #100314**

⊗ Anti-mouse CD28, clone 37.51, (LEAF™ format) **BioLegend Catalog #102112**

⊗ LEAF™ Purified anti-mouse IL-4 Antibody, clone 11B11 **BioLegend Catalog #504108**

⊗ Recombinant Mouse IL-2 (carrier-free) **BioLegend Catalog #575402**

⊗ Recombinant Mouse IL-12 (p70) (carrier-free) **BioLegend Catalog #577002**

⊗ Monensin Solution (1,000X) **BioLegend Catalog #420701**

⊗ Phorbol 12-myristate 13-acetate (PMA) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P8139**

⊗ Ionomycin calcium salt from *Streptomyces conglobatus* **Merck MilliporeSigma (Sigma-Aldrich) Catalog #I0634**



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 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 CD3 ϵ , clone 145-2C11 (3 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 1.0×10^6 /1ml/well. Culture cells for 5 days at 37°C, 5% CO₂, in the presence of anti-mouse CD28, clone 37.51 (3 μ g/mL), anti-mouse IL-4, clone 11B11 (10 μ g/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 μ g/ml ionomycin and 10 μ l 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.