Th1 Polarization of Mouse CD4+ Cells V.3

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MATERIALS

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MATERIALS

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

⊗ Ionomycin calcium salt from Streptomyces conglobatus **Sigma Aldrich Catalog #I0634**

⊗ MojoSort™ Mouse CD4 T Cell Isolation Kit **BioLegend Catalog #480005, 480006, 480033**

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

⊗ Anti-mouse CD3ε clone 145-2C11 (Ultra-LEAF™ format) **BioLegend Catalog #100339**

⊗ Anti-mouse CD28 clone 37.51 (Ultra-LEAF™ format) **BioLegend Catalog #102116**

⊗ Anti-mouse IL-4 clone 11B11 (Ultra-LEAF™ format) **BioLegend Catalog #504122**

⊗ Monensin Solution **BioLegend Catalog #420701**

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

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

- Sterile PBS
- Cell culture medium (RPMI 1640 supplemented with 10% FBS)
- Sterile 12-well plate
- Sterile 6-well plate

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 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 CD3ε, clone 145-2C11 (3μg/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 μg/mL), anti-mouse IL-4, clone 11B11 (10 μg/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 μg/ml ionomycin and 10 μl 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.