Th1 Polarization of Mouse CD4+ Cells V.3

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Materials

MATERIALS

- Phorbol 12-myristate 13-acetate (PMA) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P8139
- Ionomycin calcium salt from Streptomyces conglobatus **Merck MilliporeSigma (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

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-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 x 10^6 /1ml/well. Culture cells for 5 days at 37°C, 5% CO_2, 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.