Th9 Polarization of Mouse CD4+ Cells V.3

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Materials

MATERIALS

- 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 IFN-γ clone XMG1.2 (Ultra-LEAF™ format) **BioLegend Catalog #**505834
- Recombinant human TGF-β1 (carrier-free) **BioLegend Catalog #**580702
- Monensin Solution **BioLegend Catalog #**420701
- Recombinant mouse IL-2 (carrier-free) **BioLegend Catalog #**575402
- Recombinant mouse IL-4 (carrier-free) **BioLegend Catalog #**574302
- Sterile PBS
- Cell culture medium (IMDM supplemented with 10% FBS)
- Sterile plastic petri dishes
- PMA (Phorbol 12-myristate 13-acetate) (Cat. No. P8139 from Sigma)
- Ionomycin (Cat. No. I0634 from Sigma)

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 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 CD3ε, clone 145-2C11 (5µg/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 × 10^6/5 ml/dish. Culture cells for 3 days in the presence of anti-mouse CD28, clone 37.51 (5µg/mL), recombinant human TGF-β1 (10ng/mL), recombinant mouse IL-4 (10ng/mL), recombinant mouse IL-2 (20ng/ml), and anti-mouse IFN-γ, clone XMG1.2 (10µg/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.