Th9 Polarization of Mouse CD4+ Cells V.3

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MATERIALS

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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)

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 x 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 x 10⁶/5 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.