



Treg Polarization of Mouse CD4+ Cells

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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**

✕ Recombinant human TGF-β1 (carrier-free) **BioLegend Catalog #580702**

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

- Sterile PBS
- Cell culture medium (RPMI 1640 supplemented with 10% FBS)
- Sterile 12-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- μ m nylon cell strainer to obtain single-cell suspensions in complete 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**](#).

Treg 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.
- 5 Plate CD4⁺ cells at 1.0×10^6 /ml/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), recombinant mouse IL-2 (5ng/mL), and recombinant human TGF- β 1 (5ng/ml).
- 6 On day 3, if media is yellow, add 2 ml/well of fresh media.
- 7 On day 5, after harvesting, the cells are ready for staining.
Note: Recombinant human TGF- β is effective for stimulating mouse cells.