

Treg Polarization of Mouse CD4+ Cells

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Abstract

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Guidelines

Reagent List:

- Sterile PBS
- Cell culture medium (RPMI 1640 supplemented with 10% FBS)
- Sterile 12-well plate
- RBC Lysis Buffer (Cat. No. 420301)
- Anti-mouse CD3 ϵ , clone 145-2C11 (LEAF™ format, Cat. No. 100314)
- Anti-mouse CD28, clone 37.51, (LEAF™ format, Cat. No. 102112)
- Recombinant mouse IL-2 (carrier-free) (Cat. No. 575402)
- Recombinant human TGF- β 1 (carrier-free) (Cat. No. 580702)

Materials

RBC Lysis Buffer [420301](#) by [BioLegend](#)

Anti-mouse CD3 ϵ , clone 145-2C11 (LEAF™ format) [100314](#) by [BioLegend](#)

Anti-mouse CD28, clone 37.51, (LEAF™ format) [102112](#) by [BioLegend](#)

Recombinant mouse IL-2 (carrier-free) [575402](#) by [BioLegend](#)

Recombinant human TGF- β 1 (carrier-free) [580702](#) by [BioLegend](#)

Protocol

Isolation of CD4+ Cells From Lymph Nodes

Step 1.

Harvest lymph nodes (superficial cervical, mandibular, axillary, inguinal, and mesenteric) from mice.

Isolation of CD4+ Cells From Lymph Nodes

Step 2.

Tease lymph nodes through a sterile 70- μ m nylon cell strainer to obtain single-cell suspensions incomplete RPMI containing 10% FCS (complete medium).

Isolation of CD4+ Cells From Lymph Nodes

Step 3.

Resuspend cells in complete medium and use your favorite method to isolate CD4+ cells. Checkout [Biocompare.com](https://www.biocompare.com) to find useful kits.

Treg Polarization of CD4+ Cells**Step 4.**

On day 0, coat 12-well plate with anti-mouse CD3 ϵ , clone 145-2C11 (3 μ g/ml).

Treg Polarization of CD4+ Cells**Step 5.**

Incubate at 37°C for 2 hours. (Alternatively, incubate at 4°C overnight.)

 **DURATION**

02:00:00

Treg Polarization of CD4+ Cells**Step 6.**

Aseptically decant antibody solution from the plate.

Treg Polarization of CD4+ Cells**Step 7.**

Wash plate with sterile PBS (wash 1/3).

Treg Polarization of CD4+ Cells**Step 8.**

Wash plate with sterile PBS (wash 2/3).

Treg Polarization of CD4+ Cells**Step 9.**

Wash plate with sterile PBS (wash 3/3). Discard liquid.

Treg Polarization of CD4+ Cells**Step 10.**

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 μ g/mL), recombinant mouse IL-2 (5 ng/mL), and recombinant human TGF- β 1 (5 ng/ml).

Treg Polarization of CD4+ Cells**Step 11.**

On day 3, if media is yellow, add 2 ml/well of fresh media.

Treg Polarization of CD4+ Cells**Step 12.**

On day 5, after harvesting, the cells are ready for staining.

 **NOTES**

Kelsey Knight 09 May 2016

Recombinant human TGF- β is effective for stimulating mouse cells.