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## Treg Polarization of Mouse CD4+ Cells

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Works for me

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BioLegend

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## MATERIALS

NAME	CATALOG #	VENDOR
MojoSort™ Mouse CD4 T Cell Isolation Kit	480005, 480006, 480033	BioLegend
RBC Lysis Buffer	420301	BioLegend
Anti-mouse CD3ε clone 145-2C11 (Ultra-LEAF™ format)	100339	BioLegend
Anti-mouse CD28 clone 37.51 (Ultra-LEAF™ format)	102116	BioLegend
Recombinant human TGF-β1 (carrier-free)	580702	BioLegend
Recombinant mouse IL-2 (carrier-free)	575402	BioLegend

## MATERIALS TEXT

- 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-μm 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<sup>+</sup> 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 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.
- 5 Plate CD4<sup>+</sup> cells at 1.0 x 10<sup>6</sup>/1ml/well. Culture cells for 5 days at 37°C, 5% CO<sub>2</sub>, in the presence of anti-mouse CD28, clone 37.51 (3μg/mL), recombinant mouse IL-2 (5ng/mL), and recombinant human TGF-β1 (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- $\beta$  is effective for stimulating mouse cells.



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