

# Th17 Polarization of Mouse CD4+ Cells

BioLegend, Inc.

## Abstract

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## Guidelines

### Reagent List:

- Sterile PBS
- Cell culture medium (IMDM supplemented with 10% FBS)
- Sterile plastic petri dishes
- RBC Lysis Buffer (Cat. No. 420301)
- Anti-mouse CD3 $\epsilon$ , clone 145-2C11 (LEAF™ format, Cat. No. 100314)
- Anti-mouse CD28, clone 37.51, (LEAF™ format, Cat. No. 102112)
- Anti-mouse IFN- $\gamma$ , clone XMG1.2, (LEAF™ format, Cat. No. 505812)
- Anti-mouse IL-4, clone 11B11, (LEAF™ format, Cat. No. 504108)
- Recombinant mouse IL-6 (carrier-free) (Cat. No. 575704)
- Recombinant mouse IL-23 (carrier-free) (Cat. No. 589002)
- Recombinant human TGF- $\beta$ 1 (carrier-free) (Cat. No. 580702)
- Brefeldin A (Cat. No. 420601)
- Monensin Solution (Cat. No. 420701)
- PMA (Phorbol 12-myristate 13-acetate) (Cat. No. P8139 from Sigma)
- Ionomycin (Cat. No. I0634 from Sigma)

## Materials

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

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

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

Recombinant human TGF- $\beta$ 1 (carrier-free) [580702](#) by [BioLegend](#)

LEAF™ Purified anti-mouse IL-4 Antibody, clone 11B11 [504108](#) by [BioLegend](#)

Monensin Solution (1,000X) [420701](#) by [BioLegend](#)

Phorbol 12-myristate 13-acetate (PMA) [P8139](#) by [Sigma Aldrich](#)

Ionomycin calcium salt from Streptomyces globatus [I0634](#) by [Sigma Aldrich](#)

LEAF™ Purified anti-mouse IFN- $\gamma$  Antibody ( clone XMG1.2) [505812](#) by [BioLegend](#)

Recombinant Mouse IL-6 (carrier-free) [575704](#) by [BioLegend](#)  
Recombinant Mouse IL-23 (carrier-free) [589002](#) by [BioLegend](#)  
Brefeldin A Solution (1,000X) [420601](#) 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-µm 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<sup>+</sup> cells. Checkout [Biocompare.com](#) to find useful kits.

### Th17 Polarization of CD4+ Cells

#### Step 4.

On day 0, coat 60 × 15 mm of plastic petri dishes with anti-mouse CD3ε, clone 145-2C11 (5 µg/ml).

### Th17 Polarization of CD4+ Cells

#### Step 5.

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

 **DURATION**

02:00:00

### Th17 Polarization of CD4+ Cells

#### Step 6.

Aseptically decant antibody solution from the plate.

### Th17 Polarization of CD4+ Cells

#### Step 7.

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

### Th17 Polarization of CD4+ Cells

#### Step 8.

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

### Th17 Polarization of CD4+ Cells

#### Step 9.

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

### Th17 Polarization of CD4+ Cells

#### Step 10.

Plate CD4<sup>+</sup> cells at  $1.0 \times 10^6$  /1ml/well. Culture cells for 4 days in the presence of anti-mouse CD28,

clone 37.51 (5 µg/mL), recombinant mouse IL-6 (50 ng/mL), recombinant human TGF-β1 (1 ng/mL), recombinant mouse IL-23 (5 ng/ml), anti-mouse IL-4 (10 µg/mL), and anti-mouse IFN-γ (10 µg/mL).

#### Th17 Polarization of CD4+ Cells

##### Step 11.

On day 3, slowly add 5 ml of fresh media along with same the concentration of antibodies/cytokines as used on day 0.

#### Th17 Polarization of CD4+ Cells

##### Step 12.

On day 4, wash cells once and then restimulate in complete medium with 500 ng/ml PMA and 500 ng/mL ionomycin, in the presence of Brefeldin A (If you are looking for IL-21 production, use monensin) for 4 - 5 hours.

##### DURATION

04:00:00

#### Th17 Polarization of CD4+ Cells

##### Step 13.

After harvesting, the cells are ready for staining.

##### NOTES

**Kelsey Knight** 24 May 2016

Note: recombinant human TGF-β is effective for stimulating mouse cells.