Th9 Polarization of Mouse CD4+ Cells

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Abstract

Citation: Kelsey Miller Th9 Polarization of Mouse CD4+ Cells. **protocols.io**
dx.doi.org/10.17504/protocols.io.e2cbgaw
Published: 03 Jun 2016

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 CD3ε, clone 145-2C11 (LEAF™ format, Cat. No. 100314)
- Anti-mouse CD28, clone 37.51, (LEAF™ format, Cat. No. 102112)
- Anti-mouse IFN-γ, clone XMG1.2, (LEAF™ format, Cat. No. 505812)
- Recombinant mouse IL-2 (carrier-free) (Cat. No. 575402)
- Recombinant mouse IL-4 (carrier-free) (Cat. No. 574302)
- Recombinant human TGF-β1 (carrier-free) (Cat. No. 580702)
- Monensin Solution (Cat. No. 420701)
- PMA (Phorbol 12-myristate 13-acetate) (Cat. No. P8139 from Sigma)
- Ionomycin (Cat. No. I0634 from Sigma)

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 in IMDM 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. Check out Biocompare.com to find useful kits.

Th9 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). 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.

Th9 Polarization of CD4+ Cells:

Step 5.

Plate CD4+ cells at 10 x106/5 ml/dish. Culture cells for 3 days in the presence of anti-mouse CD28, clone 37.51 (5 µg/mL), recombinant human TGF-β1 (10 ng/mL), recombinant mouse IL-4 (10 ng/mL), recombinant mouse IL-2 (20 ng/ml), and anti-mouse IFN-γ, clone XMG1.2 (10 µg/mL).

Th9 Polarization of CD4+ Cells:

Step 6.

On day 3, wash cells once and then restimulate in complete medium with 500 ng/ml PMA and 500 ng/mL ionomycin, in the presence of monensin for 6 hours.

DURATION

06:00:00

Th9 Polarization of CD4+ Cells:

Step 7.

After harvesting, the cells are ready for staining.

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NOTES

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