Th1 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
- Sterile 6-well plate
- 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 IL-4, clone 11B11, (LEAF™ format, Cat. No. 504108)
- Recombinant mouse IL-2 (carrier-free) (Cat. No. 575402)
- Recombinant mouse IL-12 (p70) (carrier-free) (Cat. No. 577002)
- 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 CD3ε, clone 145-2C11 (LEAF™ format) 100314 by BioLegend

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

LEAF™ Purified anti-mouse IL-4 Antibody, clone 11B11 504108 by BioLegend

Recombinant Mouse IL-2 (carrier-free) 575402 by BioLegend

Recombinant Mouse IL-12 (p70) (carrier-free) 577002 by BioLegend

Monensin Solution (1,000X) 420701 by BioLegend

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

Ionomycin calcium salt from Streptomyces conglobatus I0634 by Sigma Aldrich

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+cells. Check out Biocompare.com to find useful kits.

Th1 Polarization of CD4+ Cells

Step 4.

On day 0, coat 12-well plate with anti-mouse CD3ε, clone 145-2C11 (3 µg/ml).

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Step 5.

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

DURATION

02:00:00

Th1 Polarization of CD4+ Cells

Step 6.

Aseptically decant antibody solution from the plate.

Th1 Polarization of CD4+ Cells

Step 7.

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

Th1 Polarization of CD4+ Cells

Step 8.

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

Th1 Polarization of CD4+ Cells

Step 9.

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

Th1 Polarization of CD4+ Cells

Step 10.

Plate $CD4^+$ cells at 1.0 x 10^6 /1ml/well. Culture cells for 5 days at 37°C, 5% CO_2, in the presence of anti-mouse CD28, clone 37.51 (3 µg/mL), anti-mouse IL-4, clone 11B11 (10 µg/mL), recombinant mouse IL-2 (5 ng/mL), and recombinant mouse IL-12 (10 ng/ml).

Th1 Polarization of CD4+ Cells

Step 11.

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

Th1 Polarization of CD4+ Cells

Step 12.

On day 5, wash cells once and then restimulate in complete media with 50 ng/ml PMA, 1 µg/ml ionomycin and 10 µl monensin (1000x), in a 6-well plate in incubator at 37°C for 5 hours.

DURATION

05:00:00

Th1 Polarization of CD4+ Cells

Step 13.

After harvesting, the cells are ready for staining.