Th2 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 plastic petri dishes
- Sterile T-75 culture flask
- RBC Lysis Buffer (Cat. No. 420301)
- Concanavalin A (Con A) (Sigma, Cat. No. C5275)
- Anti-mouse CD3ε, 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 mouse IL-4 (carrier-free) (Cat. No. 574302)
- Monensin Solution (Cat. No. 420701)

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

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

Monensin Solution (1,000X) 420701 by BioLegend

Recombinant Mouse IL-4 (carrier-free) 574302 by BioLegend

Concanavalin A from Canavalia ensiformis (Jack bean) C5275 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. Checkout Biocompare.com to find useful kits.

Th2 Polarization of CD4+ Cells

Step 4.

On day 0, plate $CD4^+$ cells at 30 $x10^6$ /30 ml/T-75 flask.

Th2 Polarization of CD4+ Cells

Step 5.

Culture cells for 3 days in complete RPMI containing 10% FCS, Con A (5 µg/mL), recombinant mouse IL-2 (20 ng/ml), and recombinant mouse IL-4 (50 ng/ml).

DURATION
12:00:00

Th2 Polarization of CD4+ Cells

Step 6.

On day 3, harvest the cells and wash cells once.

Th2 Polarization of CD4+ Cells

Step 7.

Seed 15 $x10^6$ cells/30 ml/T-75 flask along with recombinant mouse IL-2 (20 ng/ml) and recombinant mouse IL-4 (50 ng/ml).

Th2 Polarization of CD4+ Cells

Step 8.

On day 5, coat a 60 x 15 mm tissue culture petri dish with anti-mouse CD3ε, clone 145-2C11, 10 µg/mL in PBS, 5 ml/dish. Incubate in a 4°C refrigerator overnight.

DURATION
16:00:00

Th2 Polarization of CD4+ Cells

Step 9.

On day 6, wash the anti-mouse CD3ε pre-coated tissue culture petri dish with PBS.

Th2 Polarization of CD4+ Cells

Step 10.

Harvest the cells from the flask (that were seeded on Day 5).

Th2 Polarization of CD4+ Cells

Step 11.

Wash the cells. (wash 1/2)

Th2 Polarization of CD4+ Cells

Step 12.

Wash the cells. (wash 2/2)

Th2 Polarization of CD4+ Cells

Step 13.

Seed at 20 x10^6 /10 ml/petri dish along with 10 µl of monensin (1000x) and anti-mouse CD28, clone 37.51 (5 µg/ml).

Th2 Polarization of CD4+ Cells

Step 14.

Incubate for 6 hours at 37°C in a CO_2 incubator.

DURATION
06:00:00

Th2 Polarization of CD4+ Cells

Step 15.

After harvesting, the cells are ready for staining.