Mouse Th2 Cell Multi-Color Flow Cytometry Kit

Catalog Number: FMC012
Size: 25 Tests

Product Description

This kit contains four conjugated antibodies (and corresponding isotype controls) that can be used for single-step staining of mouse Th2 cells (1 - 5):

- IL-4 R-CFS (Affinity-purified polyclonal antibody; goat IgG)
- STAT6-APC (Clone 253906; mouse IgG_{2A})
- IL-5-PE (Clone TRFK5; rat IgG_{1})
- CD4-PerCP (Clone GK1.5; rat IgG_{2B})

The kit also contains Fixation/Permeabilization Buffer (30 mL; 1% formaldehyde, saponin, and < 0.05% sodium azide) and Permeabilization/Wash Buffer (60 mL; saponin and 0.05% sodium azide).

Intended Use

This product is designed for the flow cytometric analysis of mouse Th2 cells using four fluorochrome-conjugated antibodies.

Storage

Store at 2 - 8° C in the dark. Use within 6 months of receipt.

Precautions

- Formaldehyde is a suspected carcinogen. Avoid contact with skin, eyes, and mucous membranes, and avoid inhaling fumes. In case of contact, wash immediately with water and seek medical advice.
- Sodium azide may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

Intracellular Staining Protocol with Simultaneous Fixation/Permeabilization

1. Harvest cells of interest and wash twice in PBS or Hanks' Balanced Salt Solution (HBSS).
2. Approximately 5 x 10^5 washed cells should be resuspended in 0.5 mL of Fixation/Permeabilization Buffer and incubated at 2 - 8° C for 30 minutes. To maintain a single cell suspension, cells should be vortexed intermittently.
3. Centrifuge the cells and resuspend the pellet in 100 - 200 µL of the Permeabilization/Wash Buffer.
4. Add 10 µL of each antibody or each corresponding isotype control antibody to the cells.
5. Incubate the mixture for 30 - 45 minutes at 2 - 8° C in the dark.
6. Following the incubation, remove any excess antibody by washing the cells in 2 mL of Permeabilization/Wash Buffer. Resuspend the final cell pellet in 200 - 400 µL of PBS for flow cytometric analysis.

   Notes: *Because saponin-mediated cell permeabilization is a reversible process, it is important to keep the cells in the presence of saponin during intracellular staining. Using multiple fluorochromes requires proper flow cytometric compensation to remove the spillover fluorescence from a particular probe to a certain channel (6)*.

R&D Systems, Inc.
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Typical Data

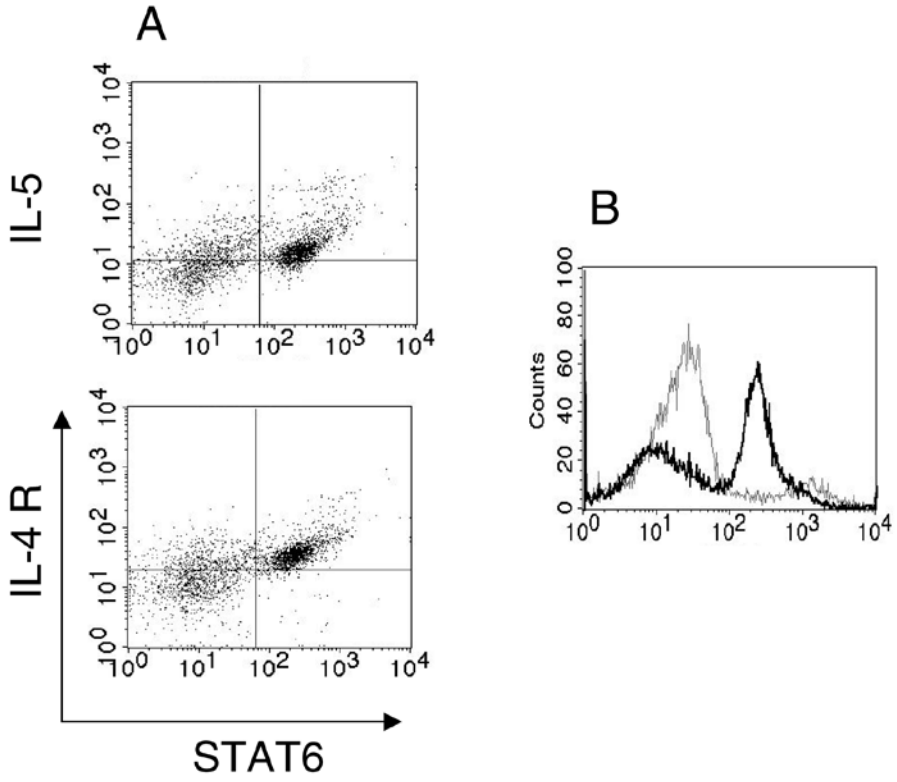

Figure 1: For Th2 activation, mouse splenocytes were cultured for 72 hours with recombinant mouse IL-4 (5 ng/mL; R&D Systems, Catalog # 404-ML), anti-mouse IFN-γ (10 μg/mL; R&D Systems, Catalog # AF-485-NA), anti-CD3ε (10 μg/mL; R&D Systems, Catalog # MAB484), and anti-CD28 (10 μg/mL; R&D Systems, Catalog # MAB4831) followed by a 3 hour re-stimulation with PMA/ionomycin. Cells were harvested and stained with the indicated antibodies following the procedure. Dot plots **(A)** show the relative IL-4 R^+, IL-5^+, and $STAT6^+$ populations from activated splenocytes on $CD4^+$-gated cells. Quadrants were set based on isotype controls. The histogram **(B)** shows the relative number of $STAT6^+$ cells in unstimulated (gray line) versus stimulated (black line) splenocytes.

References

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5. Spilianakis, C.G. and R.A. Flavell (2004) Nat. Immunol. **5**:1017.
6. Bagwell, B. and E.G. Adams (1993) Ann. N.Y. Acad. Sci. **677**:167.