



Human Th17 Cell Multi-Color Flow Cytometry Kit

Catalog Number: FMC007

Size: 25 Tests

Product Description

This kit contains four conjugated antibodies (and corresponding isotype controls) that can be used for single-step staining of human Th17 cells (1 - 11):

- IL-23 R-PE (Clone 218213; mouse IgG_{2B})
- IL-22-APC (Clone 142928; mouse IgG₁)
- IL-17-PerCP (Clone 41802; mouse IgG₁)
- CD3-CFS (Clone UCHT1; mouse IgG₁)

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

Intended Use

This product is designed for the flow cytometric analysis of Th17 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 5×10^5 washed cells should be resuspended in 0.5 mL of Fixation/Permeabilization Buffer and incubated at 2 - 8° C for 30 minutes. Cells should be vortexed intermittently in order to maintain a single cell suspension.
3. The cells are centrifuged and the pellet is resuspended in 100 - 200 μ L of the Permeabilization/Wash Buffer.
4. Add 10 μ L 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. The final cell pellet is resuspended in 200 - 400 μ L 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 (12).*

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FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

R&D Systems, Inc.

1-800-343-7475

Typical Data

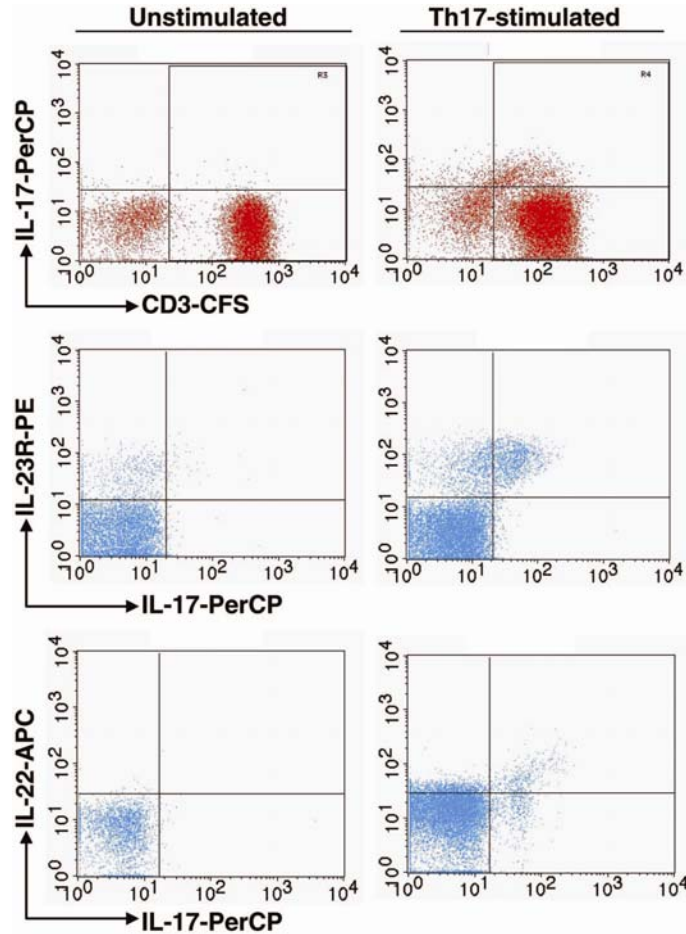


Figure 1: Human PBMCs were unstimulated (left column) or stimulated (right column) with 50 ng/mL of PMA, 200 ng/mL of ionomycin, 10 ng/mL of recombinant human IL-23 (R&D Systems, Catalog # 1290-IL), and 500 ng/mL of LPS overnight then incubated with PMA/ionomycin and 3 μ M Monesin for 2 - 4 hours and stained simultaneously with CD3, IL-17, IL-22, and IL-23 R antibodies. Dot plots shown in the top panel (CD3-CFS vs. IL-17-PerCP) were gated on lymphocytes. Subsequent dot plots were gated on CD3⁺ cells.

References

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