Mouse Th17 Cell Multi-Color Flow Cytometry Kit

Catalog Number: FMC008
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 Th17 cells (1 - 10):

- CCR6-Alexa Fluor® 488 (Clone 140706; rat IgG_{2A})
- IL-22-PE (Clone 140301; rat IgG_{2A})
- IL-17-PerCP (goat IgG)
- CD4-APC (Clone GK1.5; rat IgG_{2B})

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 mouse 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 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. Cells should be vortexed intermittently in order to maintain a single cell suspension.
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 (11)*.

Typical Data

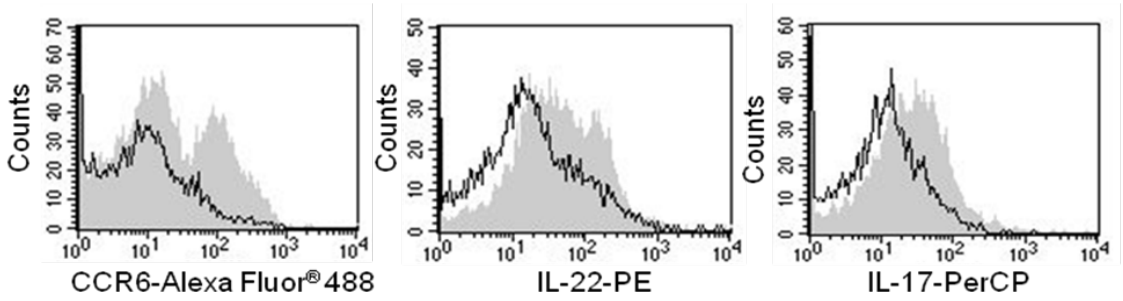

Figure 1: Mouse splenocytes were unstimulated (open histograms) or Th17-stimulated for 5 days (filled histograms), using 5 μg/mL of anti-CD3 (R&D Systems, Catalog # MAB484), 1 μg/mL of anti-CD28 (R&D Systems, Catalog # MAB4831), 10 ng/mL of recombinant mouse IL-2 (Cys160Ser) (R&D Systems, Catalog # 1150-ML), 5 ng/mL of human TGF-β (R&D Systems, Catalog # 100-B), 10 ng/mL of recombinant mouse IL-23 (R&D Systems, Catalog # 1887-ML), and 20 ng/mL of recombinant mouse IL-6 (R&D Systems, Catalog # 406-ML). Cells were harvested and stained with the antibodies in this kit or isotype controls following the procedure outlined in this insert and gated on $CD4^+$ cells. Histograms show the detected levels of the indicated antibodies in both cell samples.

References

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