Mouse Regulatory T Cell Multi-Color Flow Cytometry Kit

Catalog Number: FMC014
Size: 50 Tests

Product Description

This kit contains three conjugated antibodies (and a goat IgG-APC isotype control) that can be used for single-step staining of mouse regulatory T cells (1 - 9):

- CD25-PE (Clone 280406; rat IgG_{2A})
- CD4-PerCP (Clone GK1.5; rat IgG_{2B})
- FoxP3-APC (goat IgG)

The kit also contains Flow Cytometry FoxP3 Staining Buffer (120 mL), which contains 1% formaldehyde and 0.2% sodium azide, and Flow Cytometry Staining Buffer (50 mL), which contains BSA and 0.1% sodium azide.

Intended Use

This product is designed for the flow cytometric analysis of mouse regulatory T cells using three 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.

Staining Protocol

1. Wash mouse splenocytes (about 1 x 10^6 cells per sample) with 2 mL of PBS or Hanks' Balanced Salt Solution (HBSS), by spinning at 300 x g for 5 minutes, using 5 mL flow cytometry tubes.
2. Remove all remaining PBS or HBSS and resuspend the samples in 100 µL of Flow Cytometry Staining Buffer.
3. Add 10 µL of CD4-PerCP and 10 µL of CD25-PE antibodies or isotype controls (R&D Systems, Catalog # IC006P and IC013C).
4. Incubate the mixture for 30 - 45 minutes at 2 - 8° C in the dark.
5. Following the incubation, remove any excess antibody by washing the cells with 1 mL of Flow Cytometry FoxP3 Staining Buffer.
6. After decanting the Flow Cytometry FoxP3 Staining Buffer, add 10 µL of FoxP3-APC antibody or goat IgG-APC isotype control to the samples in the remaining small volume of buffer (about 100 µL).
7. Incubate the mixture for 1 hour at 2 - 8° C in the dark.
8. Following the incubation, remove any excess antibody by washing the cells with 1 mL of Flow Cytometry FoxP3 Staining Buffer.
9. The final cell pellet is resuspended in 200 - 400 µL of Flow Cytometry Staining Buffer for flow cytometric analysis.

Note: *Different fixation/permeabilization buffers might change side-scatter/forward-scatter patterns of the stained cells. Therefore, gating all live cells is recommended.*

R&D Systems, Inc.
1-800-343-7475

Typical Data

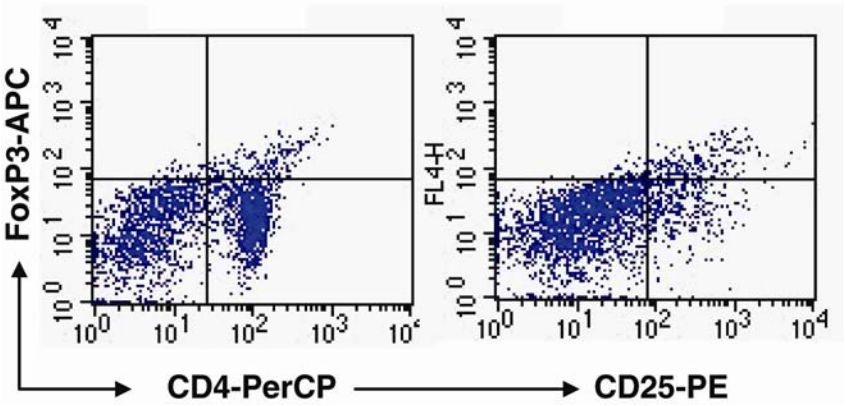

Figure 1: Mouse splenocytes were harvested and stained with the indicated antibodies following the staining protocol outlined above. Dot plots show the relative $CD4^+$, $CD25^+$, and $FoxP3^+$ populations. Quadrants were set based on isotype controls.

References

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2. Zheng, Y. and A.Y. Rudensky (2007) Nature Immunol. **8**:457.
3. Hori, S. *et al*. (2003) Science **299**:1057.
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5. Fontenot, J.D. *et al*. (2003) Nat. Immunol. **4**:330.
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