FlowX™ Human/Mouse FoxP3-PE Staining Kit

Catalog Number: FPK8214P

Sizes: 25 Tests
100 Tests

PRODUCT DESCRIPTION

This kit contains PE-conjugated Rabbit Anti-Human/Mouse FoxP3 Monoclonal Antibody and the staining buffers contained in the FlowX™ FoxP3 Fixation & Permeabilization Buffer Kit. The components of the kit have been specially formulated and optimized for immunofluorescent staining of FoxP3$^+$ single cell suspensions intended for flow cytometric acquisition and analysis.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	25-TESTS		100-TESTS		DESCRIPTION	STORAGE OF DILUTED MATERIAL
	PART #	SIZE	PART #	SIZE		
Human/Mouse FoxP3-PE Rabbit IgG, Clone 1054C	IC8214P-025	250 µL	IC8214P-100	1 mL	PE-conjugated antibody specific for human/mouse FoxP3 in a buffered protein base with preservatives.	Once diluted, components should be used and discarded.
FoxP3 Fixation Concentrate (4X)	FC012-025	8 mL	FC012-100	30 mL	A formaldehyde solution.	
FoxP3 Fixation Diluent		25 mL		100 mL	A buffered detergent.	
FoxP3 Permeabilization and Wash Buffer (10X)		25 mL		100 mL	A buffered protein base with detergent and preservatives.	

OTHER MATERIALS REQUIRED

- Blocking IgG (See Technical Hints)
- Flow Cytometry Staining Buffer (R&D Systems, Catalog # FC001)

STAINING PROTOCOL

1. Wash human PBMCs or mouse splenocytes (1 x 10^6 cells per sample) with 2 mL of Flow Cytometry Staining Buffer (R&D Systems, Catalog # FC001) or other BSA-containing buffer, by spinning at 300 x g for 5 minutes, using 5 mL flow cytometry tubes.
2. Fc-block the cells with blocking IgG (1 µg IgG/10^6 cells) for 10 minutes at 2-8 °C (See Technical Hints).
3. If cells are to be surface stained, add the desired antibody for 30 minutes at 2-8 °C.
4. Wash the cells two times with **cold** 1X PBS. During the washes, make up fresh 1X FoxP3 Fixation Buffer by diluting FoxP3 Fixation Concentrate (4X) with FoxP3 Fixation Diluent (*i.e.* 100 µL FoxP3 Fixation Concentrate (4X) + 300 µL FoxP3 Fixation Diluent).
5. Resuspend the cells in fresh 1X FoxP3 Fixation Buffer using 0.5 mL/tube. Incubate at 2-8 °C for 30 minutes. During this incubation, make up 1X FoxP3 Permeabilization and Wash Buffer by diluting FoxP3 Permeabilization and Wash Buffer (10X) with distilled water (*i.e.* 100 µL FoxP3 Permeabilization and Wash Buffer (10X) + 900 µL diH_2O) and keep at 2-8 °C.
6. Wash two times with fresh, cold, 1X FoxP3 Permeabilization and Wash Buffer.
7. Add the FoxP3 antibody to the cells (recommended concentration is 10 µL/10^6 cells) and incubate for 30 minutes at 2-8 °C.
8. Wash the cells one time with cold 1X FoxP3 Permeabilization and Wash Buffer.
9. Resuspend the cells in Flow Cytometry Staining Buffer and run on a flow cytometer.

DATA EXAMPLE

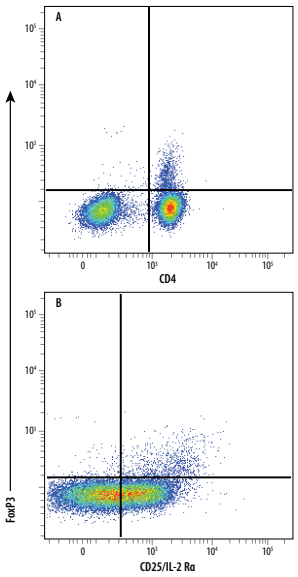

Figure 1: Detection of FoxP3 in Human PBMC Natural Regulatory T Cells (Tregs) by Flow Cytometry. Human peripheral blood mononuclear cell (PBMC) natural Tregs were surface stained with **(A)** Mouse Anti-Human CD4 Fluorescein-conjugated Monoclonal Antibody (R&D Systems, Catalog # FAB3791F) and **(B)** Mouse Anti-Human CD25/IL-2 Rα APC-conjugated Monoclonal Antibody (R&D Systems, Catalog # FAB1020A), followed by intracellular staining using Rabbit Anti-Human/Mouse FoxP3 PE-conjugated Antigen Affinity-purified Monoclonal Antibody (R&D Systems, Catalog # IC8214P). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (R&D Systems, Catalog # FC012). Cells were gated on lymphocytes.

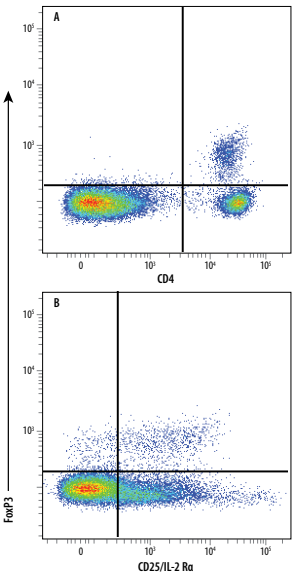

Figure 2: Detection of FoxP3 in C57/B6 Mouse Splenocyte Natural Regulatory T Cells (Tregs) by Flow Cytometry. C57/B6 mouse splenocyte natural Tregs were surface stained with **(A)** Rat Anti-Mouse CD4 APC-conjugated Monoclonal Antibody (R&D Systems, Catalog # FAB554A) and **(B)** Rat Anti-Mouse CD25/IL-2 Rα APC-conjugated Monoclonal Antibody (R&D Systems, Catalog # FAB2438A) followed by intracellular staining using Rabbit Anti-Human/Mouse FoxP3 PE-conjugated Antigen Affinity-purified Monoclonal Antibody (R&D Systems, Catalog # IC8214P). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoxP3 Fixation & Permeabilization Buffer Kit (R&D Systems, Catalog # FC012). Cells were gated on lymphocytes.

PRECAUTIONS

This product contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

This product contains formaldehyde, which 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.

TECHNICAL HINTS

- Many cells express Fc receptors (CD16, CD32, and CD64) that bind the Fc region of IgG and thus confound data interpretation. Pretreatment of cells to be stained with a variety of reagents to block Fc receptor mediated antibody interactions is always recommended. Alternatively, preincubation with excess IgG of either human, mouse or rat origin (1 µg of IgG per 1 x 10^5 cells for 15 minutes at room temperature prior to staining) will reduce unwanted Fc receptor interactions. When performing Fc receptor blocking with excess IgG while using unconjugated antibodies, it is imperative that the IgG for Fc receptor blocking not be of the same species of origin as the primary antibody to prevent the secondary antibody from recognizing any Fc receptor bound IgG.
- Setting up control reactions with appropriate isotype controls is useful in data interpretation; however, investigators should realize that different cells might interact to different degrees with isotype control reagents. Matching of immunoglobulin class and isotype, although widely used as a control reaction, may not be suitable in all staining reactions. In some cases comparing the reactivity of the primary antibody between known positive and known negative cells is an acceptable alternative to the use of isotype control reagents.