Flow Cytometry FoxP3 Staining Buffer (1X)

Catalog Number: FC011
Volume: 250 mL

Product Description

Flow Cytometry FoxP3 Staining Buffer is formulated and optimized for immunofluorescent staining of $FoxP3^+$ single-cell suspensions intended for flow cytometric acquisition and analysis. This product is supplemented with the metabolic inhibitor sodium azide.

Intended Use

This product is designed for flow cytometry applications with fluorochrome-conjugated antibodies. Refer to product specification sheets to obtain the recommended working antibody dilutions.

Stability and Storage

Upon receipt, Flow Cytometry FoxP3 Staining Buffer may be stored at 2 - 8° C for up to 6 months.

Precautions

- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- This product contains 0.2% 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 1% 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.

Staining Protocol

1. Wash human PBMCs or 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 (R&D Systems, Catalog # FC001).
3. Add 10 µL of human or mouse CD4-PerCP (R&D Systems, Catalog # FAB3791C or FAB554C) and 10 µL of human or mouse CD25-PE (R&D Systems, Catalog # FAB1020P or FAB2438P) antibodies or respective isotype controls (R&D Systems, Catalog # IC003P and IC003C or 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 (R&D Systems, Catalog # IC3240A) or goat IgG-APC isotype control (R&D Systems, Catalog # IC108A) 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 should be resuspended in 200 - 400 µL of Flow Cytometry Staining Buffer (R&D Systems, Catalog # FC001) for flow cytometric analysis.

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

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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.