Flow Cytometry Fixation/Permeabilization Buffer I (1X)

Catalog Number: FC007

PRODUCT DESCRIPTION

Flow Cytometry Fixation/Permeabilization Buffer I is formulated and optimized for simultaneous fixation and permeabilization of single-cell suspensions, dilution, and washing of these samples for subsequent intracellular flow cytometry applications.

INTENDED USE

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

STABILITY & STORAGE

Flow Cytometry Fixation/Permeabilization Buffer I (1X) may be stored at 2-8 °C for up to 6 months from date of receipt.
Note: *Color may vary. Color variance does not affect product performance.*

INTRACELLULAR STAINING PROTOCOL

1. Wash cells twice with PBS or Hanks' Balanced Salt Solution (HBSS), spinning the tube at 300 x g for 5 minutes.
2. Resuspend 5 x 10^5 washed cells in 0.5 mL of Flow Cytometry Fixation/Permeabilization Buffer I and incubate at 2-8 °C for 30 minutes. Vortex cells intermittently in order to maintain a single cell suspension.
3. Centrifuge the cells and resuspend the pellet in 100-200 µL of the Flow Cytometry Permeabilization/Wash Buffer I (R&D Systems, Catalog # FC005).
4. Add antibody to the cells at the optimal concentration.
5. Incubate the mixture for 30-45 minutes at 2-8 °C.
6. Following the incubation, remove any excess antibody by washing the cells once in 1 mL of Flow Cytometry Permeabilization/Wash Buffer I. Resuspend the final cell pellet in 200-400 µL of Flow Cytometry Staining Buffer (R&D Systems, Catalog # FC001) for flow cytometric analysis. Note: Because saponin-mediated cell permeabilization is a reversible process, it is important to keep the cells in the presence of saponin during intracellular staining.

PRECAUTIONS

The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

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.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

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TECHNICAL HINTS

- Cells must be in a single cell suspension to be analyzed by flow cytometry, therefore some cell manipulation steps may be necessary. When staining whole blood, the presence of erythrocytes can interfere with the analysis. In most cases it is advisable to lyse erythrocytes after antibody staining but prior to the flow cytometric analysis using commercially available erythrocyte lysing reagents like R&D Systems Human Lyse Buffer (Catalog # WL1000 or FC002) or R&D Systems Mouse Lyse Buffer (Catalog # WL2000 or FC003). The exceptions to the erythrocyte lysis step are as follows: a) when staining red blood cells and b) when cells are being fixed and permeabilized for intracellular staining purposes, in which case erythrocyte lysis should occur prior to antibody staining.
- Typically between 1 x 10^5 and 1 x 10^6 cells can be stained in a single reaction tube. It is important that the antibodies be used at saturating concentrations. A reasonable starting point is 1 µg of antibody per 1 x 10^6 cells being stained, although many antibodies work at much lower concentrations. There are many benefits to working with lower antibody concentrations, such as lower background staining. Since different cells may express varying levels of each antigen, customers should always titer the antibody to determine the optimal staining concentration for each antibody under their specific test conditions.
- 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.
- For intracellular staining of cells, the cells of interest must first be lightly fixed and permeabilized prior to exposure to staining reagents. Additionally, the permeabilizing solution should be used throughout the subsequent staining reaction and wash steps so that excess reagents do not remain trapped inside cells. A detailed procedure for intracellular staining is given in package inserts provided with R&D Systems Flow Cytometry Fixation Buffer (Catalog # FC004), Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005), and Flow Cytometry Fixation/Permeabilization Buffer I (Catalog # FC007).