Flow Cytometry Staining Buffer (1X)

Catalog Number: FC001
Volume: 500 mL

Product Description

Flow Cytometry Staining Buffer is formulated and optimized for immunofluorescent staining of single-cell suspensions and subsequent dilution, suspension, washing, and storage of cells intended for flow cytometric acquisition and analysis. This product is supplemented with Bovine Serum Albumin (BSA) and the metabolic inhibitor sodium azide.

Intended Use

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

Stability and Storage

Upon receipt, Flow Cytometry 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.
- Contains 0.1% sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

Surface Staining Protocol

1. Wash cells twice with 2 mL of Flow Cytometry Staining Buffer, spinning the tube at 300 x g for 5 minutes.
2. At this point, Fc receptor blocking reagents may be added to washed and counted cells. For antibodies directed to the Fc receptor follow the manufacturer's recommendations. If using excess pre-immune IgG to block Fc receptors, use 1 μg of IgG per 1 x 10^5 cells to be stained. The excess IgG does not need to be washed from the cells following the incubation period and can be carried into the staining reaction.
3. For staining, transfer cells to a 5 mL flow cytometry tube at a concentration between 1 x 10^6 and 1 x 10^7 cells per 100 μL volume.
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 twice in 2 mL of Flow Cytometry Staining Buffer. Resuspend the final cell pellet in 200 - 400 μL of Flow Cytometry Staining Buffer for flow cytometric analysis.

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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.
- 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. Treating cells with Fc receptor blocking antibodies is 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.
- 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).