Monoclonal Anti-mouse Siglec-H-PE

Catalog Number: FAB7319P
Lot Number: ACWJ01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated rat monoclonal anti-mouse Siglec-H: Supplied as 50 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 730407

Isotype: rat IgG_1

Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

Storage

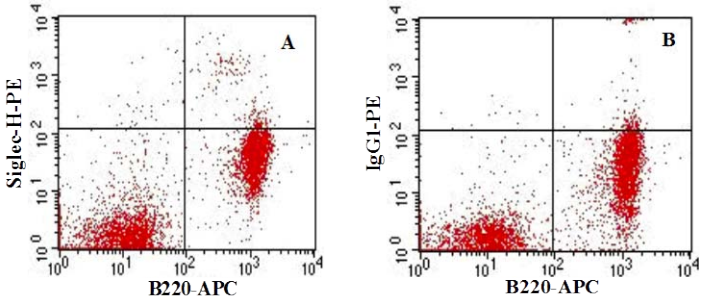
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing Siglec-H within a population and qualitatively determine the density of Siglec-H on cell surfaces by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a rat immunized with purified NS0-derived recombinant mouse Siglec-H (rmSiglec-H; aa 1-269; Accession # NP_848821). The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of Siglec-H is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565-605 nm.



Mouse splenocytes were stained with APC-conjugated anti-mouse B220/CD45R (Catalog # FAB1217A) and either A) PE-conjugated anti-mouse Siglec-H (Catalog # FAB7319P) or B) PE-conjugated isotype control (Catalog # IC005P).

Background Information

Siglec-H, a murine CD33-related siglec-like molecule and a member of the sialic acid-binding immunoglobulin (Ig)-like lectin (Siglec) family, is expressed specifically on plasmacytoid dendritic cell (pDC) precursors in the bone marrow, spleen, blood, and lymph nodes.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using mouse splenocytes.

1. Cells may be Fc-blocked with 1 μg of mouse IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2. After blocking, 10 μL of conjugated antibody was added to up to 1 x 10^6 cells and incubated for 30 minutes at room temperature.
3. Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Mouse Lyse Buffer (Catalog # FC003).
4. The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with PE-labeled rat IgG_1 antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.

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