



Miltenyi Biotec

Anti-Siglec-H antibodies mouse

Anti-Siglec-H-PE
Anti-Siglec-H-APC
Anti-Siglec-H-Biotin

130-093-508
130-093-509
130-093-510

Contents

1. Description
 - 1.1 Background information
 - 1.2 Applications
 - 1.3 Recommended antibody dilution
 - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining with Anti-Siglec-H antibodies
4. References

1. Description

Components	1 mL Anti-Siglec-H antibodies, mouse: monoclonal Anti-Siglec-H antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin.
Clone	551.3D3 (isotype: rat IgG1).
Capacity	100 tests or up to 10 ⁹ total cells.
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

The Anti-Siglec-H antibody reacts with the 34 kDa mouse sialic acid-binding immunoglobulin-like lectin (Siglec) H. Siglec-H is specifically expressed on mouse plasmacytoid dendritic cells¹—a subset of CD11c⁺ dendritic cells detected at low frequency in all lymphoid tissues, peripheral blood, and some non-lymphoid tissues. Binding of antibodies to Siglec-H inhibits type I interferon production, which can be induced in plasmacytoid dendritic cells by DNA and RNA viruses^{2–3}.

1.2 Applications

- Identification and enumeration of mouse plasmacytoid dendritic cells by flow cytometry or fluorescence microscopy.
 - ▲ **Note:** For evaluation of MACS® Separations using either Plasmacytoid Dendritic Cell Isolation Kit II, mouse (# 130-092-786) or Anti-mPDCA-1 MicroBeads, mouse (# 130-091-965), we recommend the use of Anti-mPDCA-1-APC (# 130-091-963) in combination with Anti-Ly-6C-PE (# 130-093-135).

1.3 Recommended antibody dilution

For antibody labeling of mouse cells.

Anti-Siglec-H conjugate	PE	APC	Biotin
Flow cytometry^a			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells	1:11	1:11	1:11

a) The indicated antibody dilutions are for up to 10⁷ cells/100 µL of buffer.

1.4 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
 - ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as mouse serum albumin, mouse serum, or fetal bovine serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with Anti-Siglec-H-Biotin.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Anti-Siglec-H antibody.

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5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

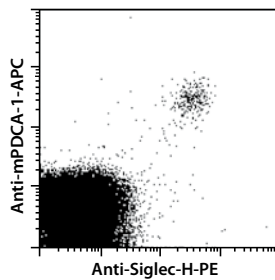
7. (Optional) If Anti-Siglec-H-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.

8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

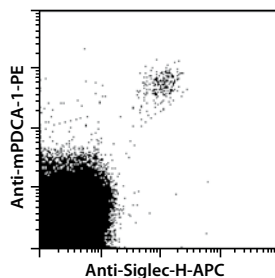
3. Examples of immunofluorescent staining with Anti-Siglec-H antibodies

Mouse spleen cells were stained with Anti-Siglec-H antibodies conjugated to PE (a) or APC (b), as well as with Anti-mPDCA-1-APC (# 130-091-963) or Anti-mPDCA-1-PE (# 130-091-962), and analyzed by flow cytometry. Cells stained with Anti-Siglec-H-Biotin (c) were stained with Anti-Biotin-APC (# 130-090-856) as well as with Anti-mPDCA-1-PE. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

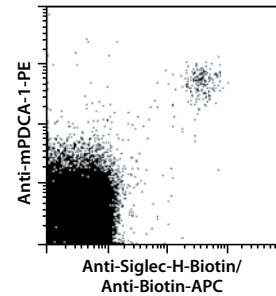
(a) Mouse BALB/c spleen cells stained with Anti-Siglec-H-PE and Anti-mPDCA-1-APC.



(b) Mouse BALB/c spleen cells stained with Anti-Siglec-H-APC and Anti-mPDCA-1-PE.



(c) Mouse BALB/c spleen cells stained with Anti-Siglec-H-Biotin, Anti-Biotin-APC, and Anti-mPDCA-1-PE.



4. References

1. Blasius, A. L. *et al.* (2006) Siglec-H is an IPC-specific receptor that modulates type I IFN secretion through DAP12. *Blood* 107(6): 2474–2476.
2. Blasius, A. *et al.* (2004) A cell-surface molecule selectively expressed on murine natural interferon-producing cells that blocks secretion of interferon-alpha. *Blood* 103(11): 4201–4206.
3. Lund, J. *et al.* (2003) Toll-like Receptor 9-mediated Recognition of Herpes Simplex Virus-2 by Plasmacytoid Dendritic Cells. *J. Exp. Med.* 198(3): 513–520.

All protocols and data sheets are available at www.miltenyibiotec.com.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

Warranty

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