Monoclonal Anti-human Clec9a-Alexa Fluor® 488

Catalog Number: FAB6049G
Lot Number: ACPV01
100 Tests

Reagents Provided

Alexa Fluor® 488-conjugated mouse monoclonal anti-human Clec9a: Supplied as 50 µg of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 683409

Isotype: mouse IgG_1

Reagents Not Provided

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

Storage

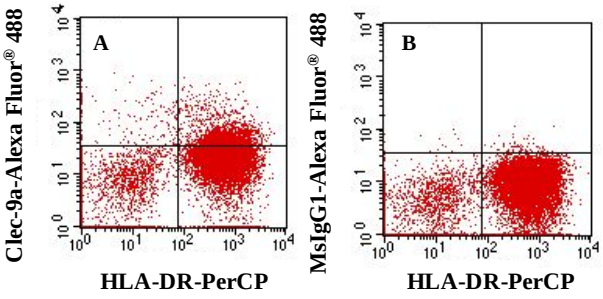
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing Clec9a within a population and qualitatively determine the density of Clec9a 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 mouse immunized with purified NS0-derived recombinant human Clec9a (aa 57-241; Accession # Q6UXN8). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to Alexa Fluor® 488 fluorochrome. Cell surface expression of Clec9a is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 515-545 nm.



Human peripheral blood cells gated on $CD3^-$ $CD141^+$ cells were stained with PerCP-conjugated anti-human HLA-DR (Catalog # FAB4869C) and **A)** Alexa Fluor® 488-conjugated anti-human Clec9a (Catalog # FAB6049G) or **B)** isotype control (Catalog # IC002G).

Background Information

CLEC9a (C-type lectin domain family 9 member A), also known as DNGR 1, is a type II transmembrane glycoprotein belonging to the C-type lectin superfamily. Although the C-type lectin domain (CTLD) of CLEC9a structurally resembles that of other C-type lectins, it lacks the conserved residues that typically mediate calcium and carbohydrate binding. CLEC9a is expressed as a disulfide-linked homodimer of approximately 50 kDa N-glycosylated subunits. Human CLEC9a expression is restricted to a subpopulation of BDCA-3^+ conventional dendritic cells (cDC) and CD16 monocytes. BDCA-3^+ cDCs are analogous to mouse $CD8^+$ cDCs which are specialized in antigenic cross-presentation in complex with MHC class I molecules. In mouse, CLEC9a is additionally expressed on plasmacytoid dendritic cells. CLEC9a ligation enhances antigen uptake and processing, leading to presentation on MHC class I and cytotoxic T cell (CTL) priming. In mouse, CLEC9a normally recognizes intracellular determinant(s) of necrotic cells and mediates their uptake by the dendritic cell. The subsequent antigenic cross-presentation to CTLs is important for clearing necrotic cellular debris. CLEC9a signaling triggers activation of the tyrosine kinase, Syk. Alternative splicing of mouse CLEC9a generates isoforms with deletions in the transmembrane segment, stalk region, or CTLD. Within amino acids (aa) 57-264 of the ECD, mouse CLEC9a shares 57% and 80% aa sequence identity with human and rat CLEC9a, respectively.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using HLA-DR+ human peripheral blood cells.

1. Cells may be Fc-blocked with 1 µg of human IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2. After blocking, 5 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 Human Lyse Buffer (Catalog # FC002).
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 Alexa Fluor® 488-labeled mouse 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.

Legal

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