

Reagents Provided

Alexa Fluor® 488-conjugated mouse monoclonal anti-human CD161:
Supplied as 25 µg of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 702228

Isotype: mouse IgG_{2B}

Reagents Not Provided

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

Storage

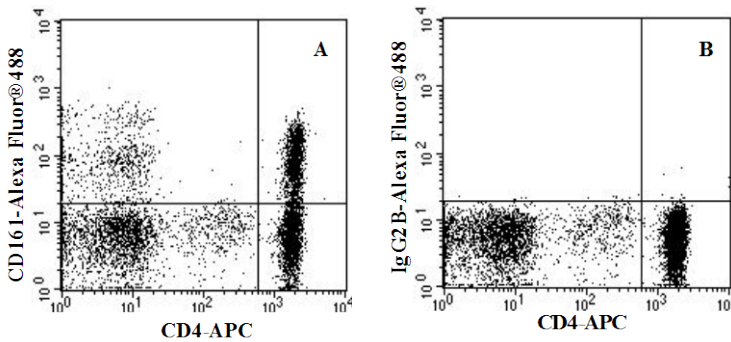
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing CD161 within a population and qualitatively determine the density of CD161 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 the BaF3 mouse pro-B cell line transfected with human CD161; (Accession # Q12918). The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to Alexa Fluor® 488 fluorochrome. Cell surface expression of CD161 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 whole blood lymphocytes were stained with APC-conjugated anti-human CD4 (Catalog # FAB3791A) and either A) Alexa Fluor® 488-conjugated anti-human CD161 (Catalog # FAB7448G) or B) Alexa Fluor® 488-conjugated isotype control (Catalog # IC0041G).

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Background Information

CD161, also known as NKR-P1A and KLRB1, is a 40 kDa type II transmembrane glycoprotein that contains one C-type lectin domain in its extracellular region. CD161 is expressed as a disulfide-linked dimer on the surface of Th17 cells and natural killer (NK) cells, as well as on subsets of CD1-restricted T cells, intestinal NT cells, peripheral memory T cells, monocytes, and dendritic cells. It binds to OCIL/CLEC2d, leading to an inhibition of NK cell-mediated cytotoxicity and IFN-γ secretion. Alternatively, CD161 can enhance TCR activation to CD1d ligation. CD161⁺ cell populations are depleted in ulcerative colitis, Grave's disease, and AIDS, although CD161⁺ T cells are activated during asthmatic attacks. Additional related proteins are expressed in mouse but not human: the inhibitory NKR-P1B and D, and the stimulatory NKR-P1A, C, and F. Human CD161 shares 47% aa sequence identity with mouse and rat NKR-P1A.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using human whole blood lymphocytes.

- Cells may be Fc-blocked with 1 µg of human IgG/10⁵ cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
- After blocking, 5 µL of conjugated antibody was added to up to 1 x 10⁶ cells and incubated for 30 minutes at room temperature.
- 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).
- 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_{2B} 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.