

Reagents Provided

Allphycocyanin (APC)-conjugated mouse monoclonal anti-human CD160: Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 688327

Isotype: mouse IgG_{2b}

Reagents Not Provided

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

Storage

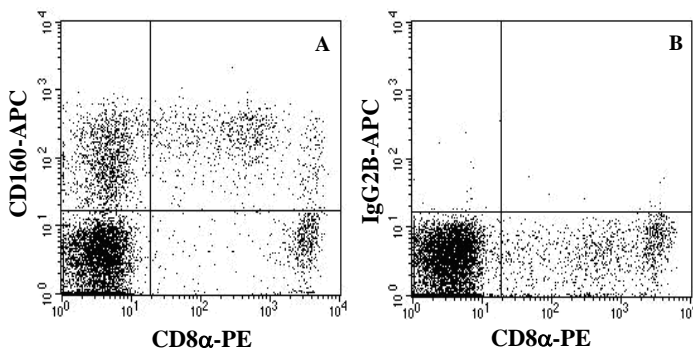
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing CD160 within a population and qualitatively determine the density of CD160 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, CHO-S-derived, recombinant human CD160 (rhCD160; aa 27 - 159; Accession # O95971) extracellular domain. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Cell surface expression of CD160 is determined by flow cytometry using 620 - 650 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.



Human peripheral blood lymphocytes were stained with A) APC-conjugated anti-human CD160 (Catalog # FAB6700A) or B) APC-conjugated isotype control (Catalog # IC0041A), followed by PE-conjugated anti-human CD8α (Catalog # FAB1509P).

Background Information

CD160 (also BY55) is a 27 kDa member of Ig superfamily of molecules. It is expressed on select hematopoietic cell types, including CD56^{dim}CD16⁺ cytotoxic NK cells, CD8⁺CD28 effector T cells, γδ T cells, and restricted CD4⁺ T cells. It is a receptor for HLAC molecules, and its engagement induces CD160⁺ NK cells to both secrete IFN-γ plus TNF-α, and initiate a cytotoxic program. Human CD160 was originally identified as a 155 amino acid (aa) pro-protein (aa 27 - 181) (SwissProt # O95971). It contains a 132 aa mature region (aa 27 - 159) and a C-terminal pro-segment that is cleaved to create a GPI linkage. The mature region possesses one V-type Ig-like domain (aa 27 - 122). CD160 is found as a soluble, disulfide-linked 80 kDa multimer (likely trimer) that is generated by proteolysis of the GPI-linked form. This 80 kDa form, plus others, are highly resistant to reduction. There is also a 100 - 110 kDa multimeric transmembrane (TM) form that is associated with activated NK cells. It contains a 55 aa substitution for Gly180Leu181, and shows a 20 aa TM segment between aa 163 - 182. The TM form appears to have a splice variant that lacks aa 25 - 133. Over aa 27 - 159, human CD160 shares only 62% aa sequence identity with mouse CD160.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using human peripheral 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, 10 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 APC-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.