

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human CCRL2/CRAM-A/B: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 152254

Isotype: mouse IgG_{2b}

Reagents Not Provided

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

Storage

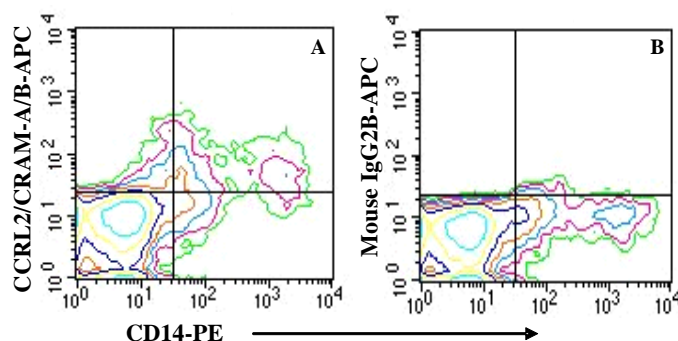
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing CCRL2/CRAM-A/B within a population and qualitatively determine the density of CCRL2/CRAM-A/B 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 CCRL2/CRAM-A/B (Accession # O00421). 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 CCRL2/CRAM-A/B 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.



PBMCs, cultured with IL-2, were stained with either A) APC-conjugated anti-human CCRL2/CRAM-A/B (Catalog # FAB23501A) or B) APC-conjugated isotype control (Catalog # IC0041A), followed by PE-conjugated anti-human CD14 (Catalog # FAB3832P) as indicated.

Background Information

CCRL2, also known as CRAM-A, CRAM-B, CKRX, and HCR, is a seven transmembrane G protein-linked receptor that shares homology with other human chemokine receptors. Two isoforms were reported and designated CRAM-A and CRAM-B that differ at their N-termini by the inclusion of an additional 12 amino acids in CRAM-A. CCRL2 is expressed at varying levels on a variety of peripheral blood cells, including monocytes, neutrophils, and T cells.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using peripheral blood mononuclear cells (PBMCs).

- 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.