

## Reagents Provided

**Carboxyfluorescein (CFS)-conjugated mouse monoclonal anti-human FCRL5/FCRL3:** Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 307307

**Isotype:** mouse IgG<sub>2b</sub>

## Reagents Not Provided

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

## Storage

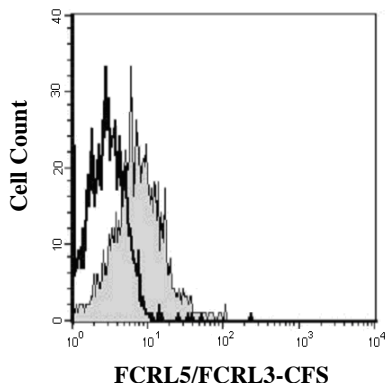
Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing FCRL5/FCRL3 within a population and qualitatively determine the density of FCRL5/FCRL3 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 NS0-derived recombinant human FCRL5/FCRL3 (rhFCRL5/FCRL3; aa 16 - 844; Accession # AAK93971). By direct ELISA, this monoclonal antibody shows 100% XR with human FCRL3. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to CFS fluorochrome. Cell surface expression of FCRL5/FCRL3 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.



CD19<sup>+</sup> human lymphocytes were stained with CFS-conjugated anti-human FCRL5/FCRL3 (Catalog # FAB20871F, filled histogram) or CFS-conjugated isotype control (Catalog # IC0041F, open histogram).

## Background Information

Fc receptor-like 5 (FCRL5), also known as FcRH5 and IRTA2, is a member of the Ig superfamily. It shares sequence homology with the classical Fc receptors for Ig and possesses cytoplasmic ITIM and ITAM-like motifs. Secreted variants and a GPI-anchored form of FCRL5 also exist. FCRL5 is expressed in naïve and memory B cells and in plasma cells. The gene for FCRL5 is localized to the human chromosome 1q21 - 23 region, a hotspot for translocation events.

## Flow Cytometry Validation

This antibody has been tested for flow cytometry using CD19<sup>+</sup> human lymphocytes.

- Cells may be Fc-blocked with 1 µg of human IgG/10<sup>5</sup> 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 1 - 2.5 x 10<sup>5</sup> 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 analysis, cells in a separate tube should be treated with CFS-labeled mouse IgG<sub>2b</sub> antibody. This procedure may need to be modified, depending upon cell type and final utilization. Individual users may need to titrate to determine 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.