

## Reagents Provided

**Allophycocyanin (APC)-conjugated rat monoclonal anti-mouse FCRL1/FcRH1:** Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

**Clone #:** 745916

**Isotype:** rat IgG<sub>1</sub>

## Reagents Not Provided

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

## Storage

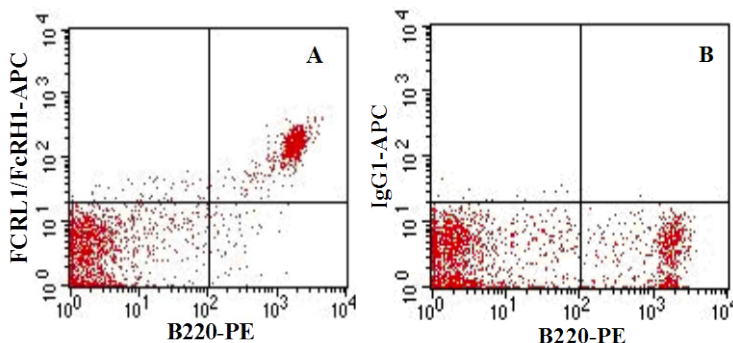
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

## Intended Use

Designed to quantitatively determine the percentage of cells bearing FCRL1/FcRH1 within a population and qualitatively determine the density of FCRL1/FcRH1 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 mouse FCRL1/FcRH1 (rmFCRL1/FcRH1; Accession # Q8R4Y0) extracellular domain. The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Cell surface expression of FCRL1/FcRH1 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.



Mouse splenocytes were stained with PE-conjugated anti-mouse B220 (Catalog # FAB1217P) and either A) APC-conjugated anti-mouse FCRL1/FcRH1 (Catalog # FAB7286A) or B) APC-conjugated isotype control (Catalog # IC005A).

## Background Information

FCRL1 (Fc receptor-like protein 1; also known as CD307a, FcRH1, and IFGP1) is a 36 kDa (predicted) member of the Ig superfamily. In mouse, it is found on mature follicular and marginal B cells, splenic T cells, and natural killer cells. It would appear that mouse FCRL1 serves as an inhibiting co-receptor, in as much as it contains a series of cytoplasmic ITIM motifs. Mature mouse FCRL1 is a 327 amino acid (aa) type I transmembrane protein (aa 17343). Over aa 17-219, mouse FCRL1 shares 83% aa sequence identity with rat FCRL1. Over the same region, it shares 63% aa sequence identity with human FCRL1 when a third Ig-like domain that is present in human is excluded from the analysis.

## Flow Cytometry Validation

This antibody has been tested for flow cytometry using mouse splenocytes.

- Cells may be Fc-blocked with 1 µg of mouse 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 up to 1 x 10<sup>6</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 Mouse Lyse Buffer (Catalog # FC003).
- 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 rat IgG<sub>1</sub> 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.