Polyclonal
Anti-mouse FCRL5/FcRH3-Phycoerythrin

Catalog Number: FAB6757P
Lot Number: ACUS01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated sheep polyclonal anti-mouse FCRL5/FcRH3: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Isotype: sheep IgG

Reagents Not Provided

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

Storage

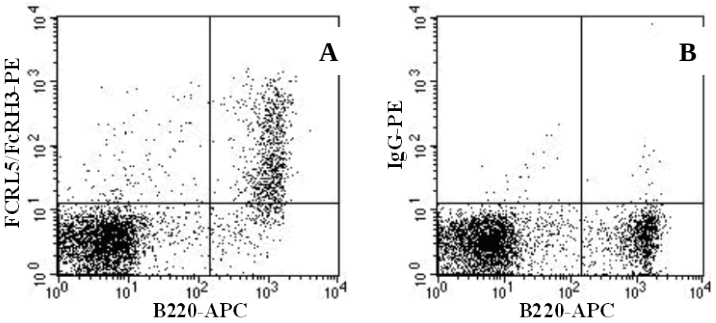
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing FCRL5/FcRH3 within a population and qualitatively determine the density of FCRL5/FcRH3 on cell surfaces by flow cytometry.

Product Description

This antibody was produced in sheep immunized with purified, NS0-derived recombinant mouse FCRL5/FcRH3 (rm FCRL5/FcRH3, Gln27Ala496 (predicted); Accession # NP_899045). Mouse FCRL5/FcRH3 specific IgG was purified by mouse FCRL5/FcRH3 affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of FCRL5/FcRH3 is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565-605 nm.



Mouse splenocytes were stained with APC-conjugated anti-mouse B220 (Catalog # FAB1217A) and either A) PE-conjugated anti-mouse FCRL5/FcRH3 (Catalog # FAB6757P) or B) PE-conjugated isotype control (Catalog # IC016P).

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.

Background Information

Fc Receptor-Like 5 (FCRL5), also known as FcRH3 (FcRH5 in human), IRTA2, and CD307e, is a 90-95 kDa member of the FCRL family of proteins whose amino acid (aa) sequence is reminiscent of classical Fc receptors. FCLR molecules are type I transmembrane proteins that contain from three to nine Immunoglobulin-like domains. They are differentially expressed within the B cell lineage, and can either promote or inhibit B cell proliferation and activation.[1-3] Mature mouse FCRL5 consists of a 470 aa extracellular domain (ECD), a 21 aa transmembrane segment, and a 79 aa cytoplasmic region. FCRL5 expression is restricted to mature B lineage cells in lymphoid tissues and blood, and is particularly noted to be expressed on T-independent marginal zone and B1 B cells.[3, 8] Its ligation inhibits signaling through the B cell antigen receptor.[9] Epstein-Barr virus transformation of B cells induces the up-regulation of surface FCRL5 by a direct effect of its EBNA2 protein on FCRL5 gene transcription.[10] FCRL5 on B cells functions as a receptor for the orthopoxvirus MHC class I-like protein OMCP.[11] Based on the literature and R&D Systems testing, both mouse and human FCRL5 will bind to purified IgG with high affinity.[5]

References

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2. Maltais, L.J. *et al*. (2006) Nat. Immunol. **7**:431.
3. Won, W-J. *et al*. (2006) J. Immunol. **177**:6815.
4. Davis, R.S. *et al*. (2004) Int. Immunol. **16**:1343.
5. Hatzivassiliou, G. *et al*. (2001) Immunity **14**:277.
6. Miller, I. *et al*. (2002) Blood **99**:2662.
7. Polson, A.G. *et al*. (2006) Int. Immunol. **18**:1363.
8. Vidal-Laliena, M. *et al*. (2005) Cell. Immunol. **236**:6.
9. Haga, C.L. *et al*. (2007) Proc. Natl. Acad. Sci. USA **104**:9770.
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11. Campbell, J.A. *et al*. (2010) J. Immunol. **185**:28.

Flow Cytometry Validation

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

1. Cells may be Fc-blocked with 1 µg of mouse IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2. After blocking, 10 µL of conjugated antibody was added to up to 1 x 10^6 cells and incubated for 30 minutes at room temperature.
3. 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).
4. 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 PE-labeled sheep IgG 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.