

Reagents Provided

Allophycocyanin (APC)-conjugated rat monoclonal anti-mouse

Notch-2: Supplied as 10 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 605724

Isotype: rat IgG₁

Reagents Not Provided

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

Storage

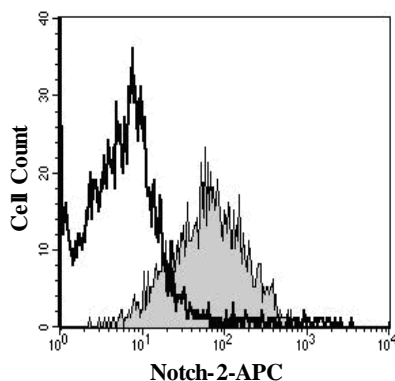
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing Notch-2 within a population and qualitatively determine the density of Notch-2 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 rat immunized with purified, CHO-derived, recombinant mouse Notch-2 (rmNotch-2; aa 1 - 528; Accession # NP_035058). 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 Notch-2 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.



C2C12 cells were stained with APC-conjugated anti-mouse Notch-2 (Catalog # FAB5196A, filled histogram) or isotype control (Catalog # IC005A, open histogram).

Background Information

Notch-2 is a 300 kDa type I transmembrane glycoprotein that is one of four Notch homologues involved in development.¹⁻³ Mouse Notch-2 is synthesized as a 2470 amino acid (aa) precursor that contains a 25 aa signal sequence, a 1652 aa extracellular domain (ECD), a 21 aa transmembrane (TM) segment, and a 772 aa cytoplasmic domain.

References

- Lardelli, M. & U. Lendahl (1993) Exp. Cell Res. **204**:364.
- Dumortier, A. *et al.* (2005) Int. J. Hematol. **82**:277.
- Yoon, K. & N. Gaiano (2005) Nat. Neurosci. **8**:709.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using C2C12 cells.

- Cells may be Fc-blocked with 1 µg of mouse 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 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 IgG₁ 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.