

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human

Notch-1: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 527425

Isotype: mouse IgG₁

Reagents Not Provided

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

Storage

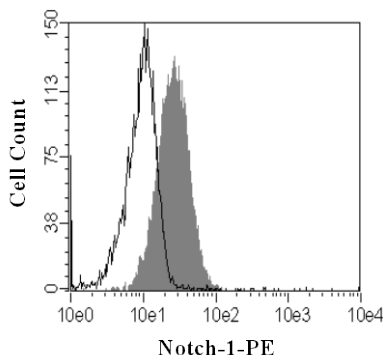
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing Notch-1 within a population and qualitatively determine the density of Notch-1 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 Notch-1 (rhNotch-1; aa 19 - 526; Accession # P46531). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of Notch-1 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.



U2OS cells were stained with PE-conjugated anti-human Notch-1 (Catalog # FAB5317P, filled histogram) or PE-conjugated isotype control (Catalog # IC002P, open histogram).

Background Information

Notch-1 (so named for “notches” in fly wings; also TAN-1) is a 300 kDa, 2538 amino acid (aa) member of the Notch family of type I TM glycoproteins. It is associated with gene activation in both embryo and adult. It undergoes Golgi processing to generate a heterodimer composed of a 180 - 200 kDa disulfide-linked extracellular domain (aa 18 - 1664), and a 120 kDa membrane-bound segment (aa 1665 - 2556) that is cleaved to form the notch intracellular domain transcription factor upon ligand binding. Human Notch-1 ECD (aa 19 - 526), including the first 13 EGF repeats, shows 91% aa identity with corresponding regions of mouse and rat Notch-1, and 60% aa identity with human Notch-2 and Notch-3.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using U2OS cells.

- 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 analysis by flow cytometry. As a control for this analysis, cells in a separate tube should be treated with PE-labeled mouse IgG₁ 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.