

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-mouse

Notch-1: Supplied as 50 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: n1A

Isotype: mouse IgG₁

Reagents Not Provided

Flow Cytometry Fixation Buffer (Catalog # FC004) or other 4% paraformaldehyde fixation buffer.

Flow Cytometry Permeabilization/Wash Buffer I (1X) (Catalog # FC005) or other saponin-containing saline buffer.

Storage

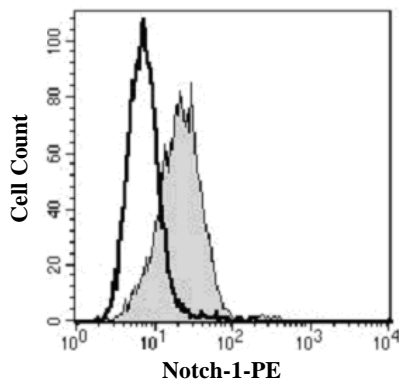
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells containing Notch-1 within a population and qualitatively determine the density of intracellular Notch-1 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 mouse Notch 1 GST fusion protein. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to a PE fluorochrome. Intracellular 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.



RPMI8226 cells were stained with PE-conjugated anti-mouse Notch-1 (Catalog # IC5267P, filled histogram) or isotype control (Catalog # IC002P, open histogram).

試薬に用いたR&D Systemsの製品は、品質を確保するために厳格に管理されています。R&D Systems, Inc. 1-800-343-7475

2009/10/02

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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 transmembrane glycoproteins. It is associated with gene activation during both embryonic development and in adult organisms. It undergoes Golgi processing to generate a heterodimer composed of a 180 - 200 kDa disulfide-linked extracellular domain, and a 120 kDa membrane-bound segment that is cleaved to form the notch intracellular domain transcription factor upon ligand binding. Mouse Notch-1 ECD (aa 19 - 526), including the first 13 EGF repeats, shares 91% and 95% aa identity with the corresponding regions of human and rat Notch-1, respectively, and 55 - 58% aa identity with mouse Notch-2 and Notch-3.

Flow Cytometry Validation

For intracellular staining, cells must first be fixed and permeabilized. We recommend the use of 4% PFA as a fixative and a 0.1% saponin balanced salt solution for permeabilization and washing (see [Reagents Not Provided](#)).

1. Cells were harvested and washed twice in saline buffer.
2. Cell surface staining may be done at this point following the manufacturer’s staining procedure.
3. 5×10^5 cells were resuspended in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubated at room temperature for 10 minutes.
4. Following fixation, the cells were washed twice in saline buffer, then once in Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005).
5. After permeabilization, 10 µL of conjugated antibody was added and the cells were incubated for 30 minutes at room temperature **in the dark**.
6. The cells were washed twice with Flow Cytometry Permeabilization/Wash Buffer I.
7. The cells were resuspended in saline buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with PE-labeled mouse IgG, antibody. This procedure may need to be modified, depending on 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.