

Reagents Provided

Phycoerythrin (PE)-conjugated rat monoclonal anti-mouse NCAM-L1:

Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone #: 555

Isotype: rat IgG_{2A}

Reagents Not Provided

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

Storage

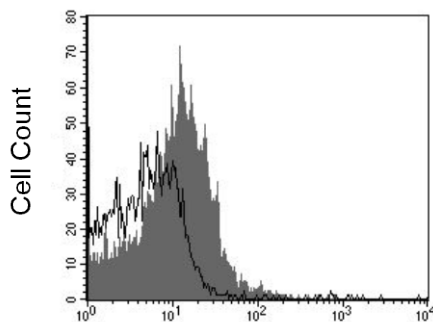
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing NCAM-L1 within a population and qualitatively determine the density of NCAM-L1 on cell surfaces by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a rat myeloma with B cells obtained from a rat immunized with an affinity-purified glycoprotein fraction from cerebella of 8- to 10-day old C57/BL6 mice (Rathjen, F.G. & M. Schachner (1984) EMBO 3:1-10). 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 NCAM-L1 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.



NCAM-L1-PE

Mouse splenocytes were stained with PE-conjugated anti-mouse NCAM-L1 (Catalog # FAB5674P, filled histogram) or isotype control (Catalog # IC006P, open histogram).

Background Information

The Neural Cell Adhesion Molecule L1 (NCAM-L1/CD171), also called L1-CAM or L1, is an Immunoglobulin superfamily (IgSF) adhesion molecule critical for neural development. NCAM-L1 binds homophilically and heterophilically with adhesion molecules including Axonin-1, CD9, Neurocan, and Integrins. Mutations of the gene encoding NCAM-L1 are associated with a subset of X-linked mental retardation syndromes, schizophrenia, and other neurological disorders. Cancer cells can proteolytically cleave NCAM-L1, releasing the soluble ectodomain. Within the ECD, human NCAM-L1 shares 86% and 87% amino acid identity with mouse and rat NCAM-L1, respectively.

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/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 analysis by flow cytometry. As a control for this analysis, cells in a separate tube should be treated with PE-labeled rat IgG_{2A} 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.