

Reagents Provided

Allophycocyanin (APC)-conjugated mouse monoclonal anti-human Nectin-2/CD112: Supplied as 25 µg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 610603

Isotype: mouse IgG₁

Reagents Not Provided

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

Storage

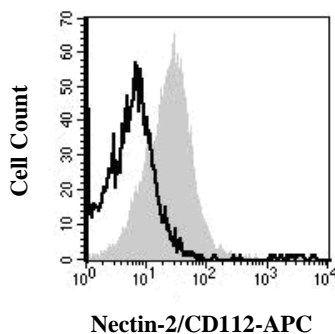
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing Nectin-2/CD112 within a population and qualitatively determine the density of Nectin-2/CD112 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 Nectin-2/CD112 (rhNectin-2/CD112; aa 32 - 360; Accession # NP_002847) extracellular domain. The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to APC fluorochrome. Cell surface expression of Nectin-2/CD112 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.



K562 cells were stained with APC-conjugated anti-human Nectin-2/CD112 (Catalog # FAB2229A, filled histogram) or APC-conjugated isotype control (Catalog # IC002A, open histogram).

Background Information

Nectins are a small family of Ca²⁺-independent immunoglobulin (Ig)-like cell adhesion molecules (CAMs) that organize intercellular junctions.¹ The nectin family has at least four members that are highly homologous to the human receptor for poliovirus. Nectin-2/CD112 is a 60 or 65 kDa type I transmembrane glycoprotein that is found on a variety of cell types,^{2,3} and is known to bind the pseudorabies virus and herpes simplex virus2 (HSV2), but not HSV1 or poliovirus.

References

- Takai, Y. & H. Nakanishi (2003) J. Cell Sci. **116**:17.
- Bottino, C. *et al.* (2003) J. Exp. Med. **198**:557.
- Pende, D. *et al.* (2005) Mol. Immunol. **42**:463.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using K562 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 final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with APC-labeled mouse 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.