

Monoclonal Anti-human N-Cadherin Propeptide-Phycoerythrin

Catalog Number: FAB1388P

Lot Number: AAOG02

100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated rat monoclonal anti-human

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

Clone #: 401408

Isotype: rat IgG_{2A}

Reagents Not Provided

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

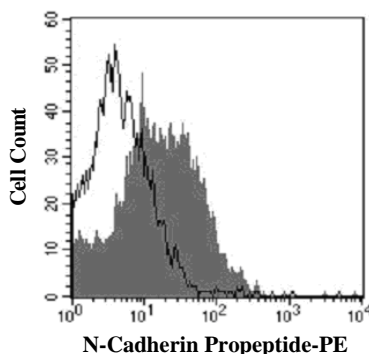
Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing N-Cadherin within a population and qualitatively determine the density of N-Cadherin 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, *E. coli*-derived, recombinant human N-Cadherin propeptide (rhN-Cadherin; aa 26 - 159; Accession # P19022). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Expression of N-Cadherin 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.



HEK293 cells were stained with PE-conjugated anti-human N-Cadherin Propeptide (Catalog # FAB1388P, filled histogram) or isotype control (Catalog # IC006P, open histogram).

Background Information

Neuronal Cadherin (N-Cadherin or NCAD), also named Cadherin 2 (CDH2) is a type I membrane protein belonging to the Cadherin superfamily of Ca²⁺-dependent adhesion molecules.¹⁻³ N-Cadherin, together with E-, P-, R-, and M-Cadherins, constitute the type I classic Cadherin subfamily. Members of this subfamily are synthesized with a propeptide sequence,⁴ five extracellular Cadherin repeats with a conserved HAV motif in the N-terminal Cadherin domain, and a highly conserved cytoplasmic region that interacts with the actin cytoskeleton via catenins. It was reported that proteolytic cleavage of the propeptide is required for the proper cell surface expression of NCAD.⁴ NCAD expression is relatively ubiquitous during early vertebrate development. In adult tissues, NCAD is highly expressed in neural tissues, skeletal and cardiac muscle.

References

1. Hirano, S. *et al.*, 2003, *Frontiers in Bioscience* **8**:d306.
2. Friedel, N., *et al.*, 2000, *J. Mol. Biol.* **299**:551 - 572.
3. Redies, C., *et al.*, 1993, *Comp. Neurol.* **333**:398 - 416.
4. Wahl, J.K. III, *et al.*, 2003, *J. Biol. Chem.* **278**:17269 - 17276.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using HEK293 cells. For intracellular staining, cells must first be fixed and permeabilized. We recommend the use of 4% PFA and 0.1% saponin balanced salt solution for permeabilization and washing.

1. To 1 - 5 x 10⁵ fresh, or fixed and permeabilized cells, 10 µL of conjugated antibody was added and incubated for 30 minutes at room temperature.
2. Unbound antibody was removed by washing fresh cells twice in Flow Cytometry Staining Buffer (Catalog # FC001), or fixed and permeabilized cells twice in Flow Cytometry Permeabilization/Wash Buffer I (1X) (Catalog # FC005). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Human Lyse Buffer (Catalog # FC002).
3. The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for 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.