Polyclonal
Anti-human N-Cadherin-Alexa Fluor® 488

Catalog Number: FAB6426G
Lot Number: ACJQ01
100 Tests

Reagents Provided

Alexa Fluor® 488-conjugated-conjugated sheep polyclonal anti-human N-Cadherin: Supplied as 10 μg of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Isotype: sheep IgG

Reagents Not Provided

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

Storage

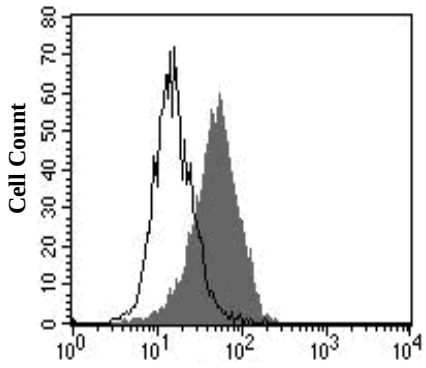
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

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

Product Description

Sheep antibodies were raised against purified NS0-derived recombinant human N-Cadherin extracellular domain (rhN-Cadherin; aa 160-724; Accession # P19022). Polyclonal antibody was affinity-purified on a column derivatized with the recombinant protein. The purified antibody was then conjugated to Alexa Fluor® 488 fluorochrome. Cell surface 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 515-545 nm.



N-Cadherin-Alexa Fluor® 488

HeLa cells were stained with Alexa Fluor® 488-conjugated anti-human N-Cadherin (Catalog # FAB6426G; filled histogram) or Alexa Fluor® 488-conjugated isotype control (Catalog # IC016G; open histogram).

Background Information

N-Cadherin (Neural Cadherin; also CD325 and Cadherin-2) is a 130-135 kDa member of the “classical” (or type I) cadherin subfamily of cadherin superfamily proteins. It is expressed on multiple cell types, including neurons, fibroblasts, Schwann cells, endothelial cells, and hepatic stellate cells. N-Cadherin mediates homotypic binding, either in *cis* (same cell) or *trans* (adjacent cell). Pro-N-Cadherin is expressed as an 881 amino acid (aa) type I transmembrane glycoprotein. It may be initially inserted into the ER, where the 15-20 kDa pro-domain (aa 26-159) is cleaved by pro-protein convertase, and the mature molecule (aa 160-906) is transported to the surface. Mature N-Cadherin contains a 565 aa extracellular region (aa 160-724) that possesses five cadherin domains (aa 160-714), and a 161 aa cytoplasmic tail that undergoes phosphorylation at Tyr785. There is one splice variant that contains a 10 aa substitution for aa 839-906. Over aa 160-724, human N-Cadherin shares 98% aa identity with mouse N-Cadherin.

Flow Cytometry Validation

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

1. Cells may be Fc-blocked with 1 μg of human IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2. After blocking, 5 μL of conjugated antibody was added to up to 1 x 10^6 cells and incubated for 30 minutes at room temperature.
3. 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 # FC003).
4. 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 Alexa Fluor® 488-labeled sheep IgG 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.

Legal