Human/Mouse/Rat N-Cadherin Antibody

Antigen Affinity-purified Polyclonal Sheep IgG

Catalog Number: AF6426

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat N-Cadherin in Western blots. In direct ELISA, less than 10% cross-reactivity with recombinant human (rh) E-Cadherin, and rhR-Cadherin is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human N-Cadherin Asp160-Ala724 Accession # P19022
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

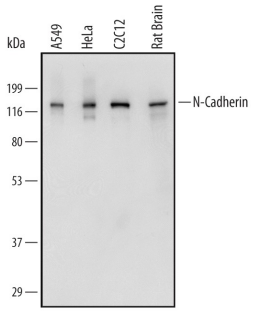
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Flow Cytometry	2.5 µg/10^6 cells	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
ELISA	This antibody functions as an ELISA detection antibody to detect human N-Cadherin when paired with Mouse Anti-Human N-Cadherin Monoclonal Antibody (Catalog # MAB13883). *This product is intended for assay development on various assay platforms requiring antibody pairs. We recommend the Human N-Cadherin DuoSet ELISA Kit (Catalog # DY1388-05) for convenient development of a sandwich ELISA.*	

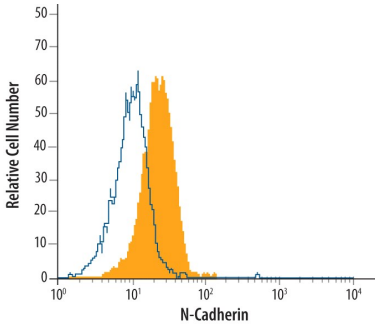
DATA

Western Blot



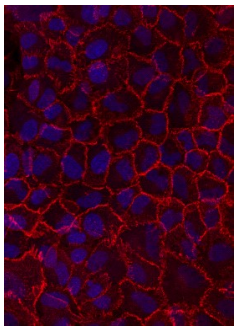
Detection of Human, Mouse, and Rat N-Cadherin by Western Blot. Western blot shows lysates of A549 human lung carcinoma cell line, HeLa human cervical epithelial carcinoma cell line, C2C12 mouse myoblast cell line, and rat brain tissue. PVDF Membrane was probed with 0.5 µg/mL of Sheep Anti-Human/Mouse/Rat N-Cadherin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6426) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for N-Cadherin at approximately 130 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



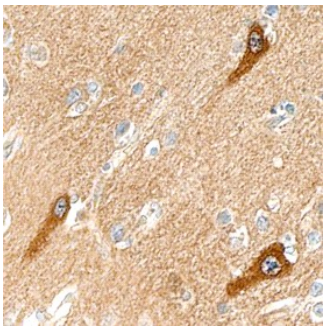
Detection of N-Cadherin in HeLa Human Cell Line by Flow Cytometry. HeLa human cervical epithelial carcinoma cell line was stained with Sheep Anti-Human/Mouse/Rat N-Cadherin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6426, filled histogram) or Sheep IgG control antibody (Catalog # 5-001-A, open histogram), followed by NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # NL010).

Immunocytochemistry



N-Cadherin in A549 Human Cell Line. N-Cadherin was detected in immersion fixed A549 human lung carcinoma cell line using Sheep Anti-Human/Mouse/Rat N-Cadherin Affinity-purified Polyclonal Antibody (Catalog # AF6426) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to cell surfaces. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



N-Cadherin in Human Brain. N-Cadherin was detected in immersion fixed paraffin-embedded sections of human brain (cortex) using Sheep Anti-Human/Mouse/Rat N-Cadherin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6426) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to neuronal cell bodies and processes. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

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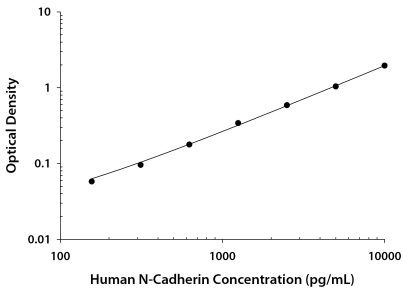
ELISA



Human N-Cadherin ELISA Standard Curve. Recombinant Human N-Cadherin protein was serially diluted 2-fold and captured by Mouse Anti-Human N-Cadherin Monoclonal Antibody (Catalog # MAB13883) coated on a Clear Polystyrene Microplate (Catalog # DY990). Sheep Anti-Human/Mouse/Rat N-Cadherin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6426) was biotinylated and incubated with the protein captured on the plate. Detection of the standard curve was achieved by incubating Streptavidin-HRP (Catalog # DY998) followed by Substrate Solution (Catalog # DY999) and stopping the enzymatic reaction with Stop Solution (Catalog # DY994).

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

N-Cadherin (Neural Cadherin; also CD325 and Cadherin-2) is a 130-135 kDa member of the "classical" (or type I) cadherin subfamily, cadherin superfamily of 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). proN-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 prodomain (aa 26-159) is cleaved by proprotein 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.

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