Human/Mouse NCAM-1/CD56 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF2408

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human NCAM-1/CD56 in direct ELISAs and Western blots. In direct ELISAs and Western blots, less than 1% cross-reactivity with recombinant human (rh) ALCAM, rhBCAM and rhEpCAM is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human NCAM1/CD56 Leu20-Pro603 Accession # NP_001070150
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.

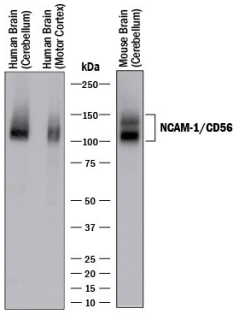
APPLICATIONS

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	0.25 µg/10^6 cells	Human peripheral blood mononuclear cells
Immunocytochemistry	5-15 µg/mL	See Below
Simple Western	5-25 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.	

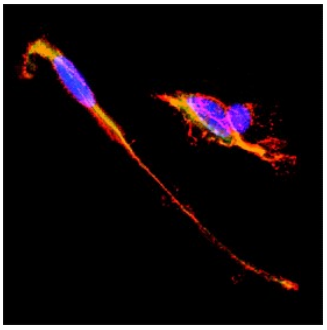
DATA

Western Blot



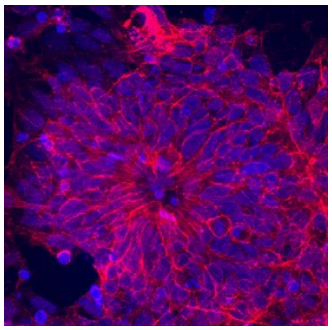
Detection of Human and Mouse NCAM-1/CD56 by Western Blot. Western blot shows lysates of human brain (cerebellum and motor cortex) tissue and mouse brain (cerebellum) tissue. PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human/Mouse NCAM-1/CD56 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2408) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). Specific bands were detected for NCAM-1/CD56 at approximately 100-150 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



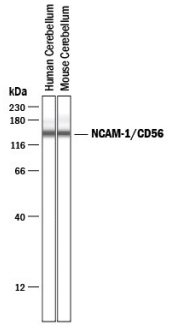
NCAM-1/CD56 in SH-SY5Y Human Neuroblastoma Cells. SH-SY5Y human neuroblastoma cells were cultured overnight in the presence of 1 mM Retinoic Acid (Catalog # 0695/50) prior to immersion fixation. Neural Cell Adhesion Molecule 1 (NCAM-1)/CD56 was detected using a Goat Anti-Human/Mouse NCAM-1/CD56 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2408). The cells were stained with the NorthernLights 557-conjugated Donkey Anti-Goat IgG Affinity-purified Secondary Antibody (red; Catalog # NL001). Actin filaments were stained with FITC-conjugated Phalloidin (green) and cell nuclei were counterstained with DAPI (blue). NCAM-1/CD56 immunoreactivity was localized to the plasma membrane. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunocytochemistry



NCAM-1/CD56 in BG01V Human Embryonic Stem Cells. NCAM-1/CD56 was detected in immersion fixed BG01V human embryonic stem cells differentiated into neural progenitor cells using Goat Anti-Human/Mouse NCAM-1/CD56 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2408) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Stem Cells on Coverslips.

Simple Western



Detection of Human and Mouse NCAM-1/CD56 by Simple Western™. Simple Western lane view shows lysates of human and mouse brain (cerebellum) tissue, loaded at 0.2 mg/mL. A specific band was detected for NCAM-1/CD56 at approximately 143 kDa (as indicated) using 5 µg/mL for human lysates and 25 µg/mL for mouse lysates of Goat Anti-Human/Mouse NCAM-1/CD56 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2408) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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Catalog Number: AF2408

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
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

Neural cell adhesion molecule 1 (NCAM-1) is a multifunctional member of the Ig superfamily. It belongs to a family of membrane-bound glycoproteins that are involved in Ca^{++} independent cell matrix and homophilic or heterophilic cell-cell interactions. NCAM-1 specifically binds to heparan sulfate proteoglycans (1), the extracellular matrix protein agrin (2), and several chondroitin sulfate proteoglycans that include neurocan and phosphocan (3). There are three main forms of human NCAM-1 that arise by alternate splicing. These are designated NCAM-120/NCAM-1 (761 amino acids [aa]), NCAM-140 (848 aa), and NCAM-180 (1120 aa). NCAM-120 is GPI-linked, while NCAM-140 and NCAM-180 are type I transmembrane glycoproteins (4-6). Additional alternate splicing adds considerable diversity to all three forms, and extracellular proteolytic processing is possible for NCAM-180 (7-8). NCAM-1 is synthesized as a 761 aa preproprecursor that contains a 19 aa signal sequence, a 722 aa GPI-linked mature region, and a 20 aa C-terminal prosegment (4). The molecule contains five C-2 type Ig-like domains and two fibronectin type-III domains. Human to mouse, NCAM-1 is 93% aa identical. NCAM-1 appears to be highly sialylated. The polysialyation of NCAM-1 reduces its adhesive property and increases its neurite outgrowth promoting features (9). NCAM-1 in the adult brain shows a decline of sialylation relative to earlier developmental periods. In regions that retain a high degree of neuronal plasticity, however, the adult brain continues to express polysialylation-NCAM-1, suggesting sialylation of NCAM-1 is involved in regenerative processes and synaptic plasticity (10-13).

References:

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USA TEL 800 343 7475 **Canada** TEL 855 668 8722 **China** TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449