

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Neuropilin-1 in direct ELISAs and Western blots. In direct ELISAs, less than 50% cross-reactivity with recombinant rat Neuropilin-1 and recombinant mouse Neuropilin-1 is observed, and less than 1% cross-reactivity with recombinant human Neuropilin-2 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human Neuropilin-1 Phe22-Lys644 Accession # NP_001019799
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
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.

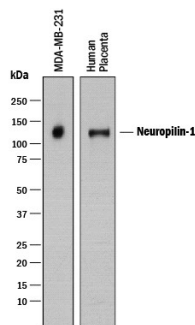
APPLICATIONS

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	1 µg/mL	See Below
Flow Cytometry	2.5 µg/10 ⁶ cells	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.	
Blockade of Receptor-ligand Interaction	In a functional ELISA, 0.25-1 µg/mL of this antibody will block 50% of the binding of 20 ng/mL of Recombinant Human VEGF (Catalog # 293-VE) to immobilized Recombinant Human Neuropilin-1 (Catalog # 3870-N1) coated at 0.75 µg/mL (100 µL/well). At 5 µg/mL, this antibody will block >90% of the binding.	

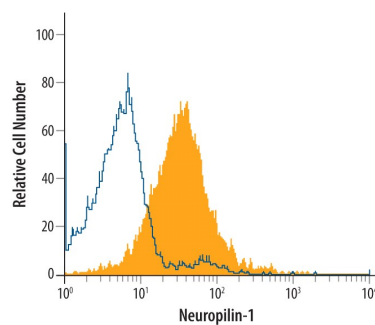
DATA

Western Blot



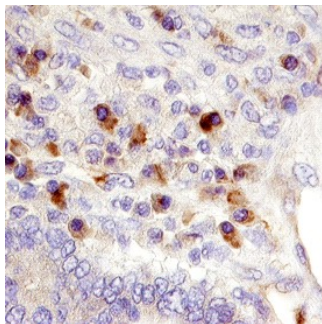
Detection of Human Neuropilin-1 by Western Blot. Western blot shows lysates of MDA-MB-231 human breast cancer cell line and human placenta tissue. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human Neuropilin-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3870) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Neuropilin-1 at approximately 130 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



Detection of Neuropilin-1 in HUVEC Human Cells by Flow Cytometry. HUVEC human umbilical vein endothelial cells were stained with Sheep Anti-Human Neuropilin-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3870, filled histogram) or control antibody (Catalog # 5-001-A, open histogram), followed by NorthernLights™ 637-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # NL011).

Immunohistochemistry



Neuropilin-1 in Human Pancreatic Cancer Tissue. Neuropilin-1 was detected in immersion fixed paraffin-embedded sections of human pancreatic cancer tissue using Sheep Anti-Human Neuropilin-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3870) at 10 µg/mL overnight at 4 °C. Before incubation with the primary antibody tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific labeling was localized to the cytoplasm and plasma membrane of cancer cells. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

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	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> ● 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

Neuropilin-1 (Npn-1, previously neuropilin; also CD304/BDCA4) is a 130-140 kDa type I transmembrane (TM) glycoprotein that regulates axon guidance and angiogenesis (1-4). The full-length 923 amino acid (aa) human Npn-1 contains a 623 aa extracellular domain (ECD) that shows 92-95% aa identity with mouse, rat, bovine, and canine Npn-1 (3, 4). The ECD contains two N-terminal CUB domains (termed a1a2), two domains with homology to coagulation factors V and VIII (b1b2) and a MAM (meprin) domain (c). C-terminally divergent splice variants with 704, 644, 609, and 551 aa lack the MAM and TM domains and are demonstrated or presumed to be soluble antagonists (1, 5-7). A 906 aa form lacks a TM segment, but secretion has not been found (8). The sema domains of Class III secreted semaphorins such as Sema3A bind Npn-1 a1a2 (9). Heparin, the heparin-binding forms of VEGF (VEGF₁₆₅, VEGF-B, and VEGF-E), PIGF (PIGF-2), and the C-terminus of Sema3 bind the b1b2 region (9, 10). Npn-1 and Npn-2 share 48% aa identity within the ECD and can form homo- and hetero-oligomers via interaction of their MAM domains (1). Neuropilins show partially overlapping expression in neuronal and endothelial cells during development (1, 2). Both neuropilins act as co-receptors with plexins, mainly plexin A3 and A4, to bind class III semaphorins that mediate axon repulsion (11). However, only Npn-1 binds Sema3A, and only Npn-2 binds Sema3F (1). Both are co-receptors with VEGF R2 (also called KDR or Flk-1) for VEGF₁₆₅ binding (1). Sema3A signaling can be blocked by VEGF₁₆₅, which has higher affinity for Npn-1 (12). Npn-1 is preferentially expressed in arteries during development or those undergoing remodeling (1, 2). Npn-1 is also expressed on dendritic cells and mediates DC-induced T cell proliferation (13).

References:

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PRODUCT SPECIFIC NOTICES

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U.S. Patent # 6,054,293, 6,623,738, and other U.S. and international patents pending.