

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat Neuropilin-2 in Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human Neuropilin-2 Gln23-Tyr855 Accession # Q7LBX6
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.

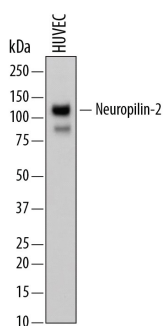
APPLICATIONS

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	0.5 µg/mL	See Below
Flow Cytometry	0.25 µg/10 ⁶ cells	HUVEC human umbilical vein endothelial cells
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	10 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, 1-3 µg/mL of this antibody will block 50% of the binding of 500 ng/mL of Recombinant Human VEGF ₁₆₅ (Catalog # 293-VE) to immobilized Recombinant Human Neuropilin-2 Fc Chimera (Catalog # 2215-N2) coated at 5 µg/mL (100 µL/well). At 20 µg/mL, this antibody will block >90% of the binding.	

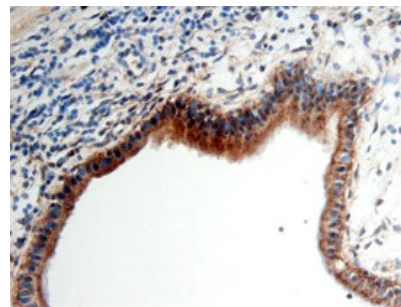
DATA

Western Blot



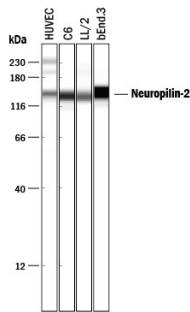
Detection of Human Neuropilin-2 by Western Blot. Western blot shows lysates of HUVEC human umbilical vein endothelial cells. PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human/Mouse/Rat Neuropilin-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2215) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). A specific band was detected for Neuropilin-2 at approximately 120 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



Neuropilin-2 in Human Pancreatic Cancer Tissue. Neuropilin-2 was detected in immersion fixed paraffin-embedded sections of human pancreatic cancer tissue using Goat Anti-Human/Mouse/Rat Neuropilin-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2215) at 5 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-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Simple Western



Detection of Human, Mouse, and Rat Neuropilin-2 by Simple Western™.

Simple Western lane view shows lysates of HUVEC human umbilical vein endothelial cells, C6 rat glioma cell line, LL/2 mouse Lewis lung carcinoma cell line, and bEnd.3 mouse endothelioma cell line, loaded at 0.2 mg/mL. A specific band was detected for Neuropilin-2 at approximately 140 kDa (as indicated) using 10 µg/mL of Goat Anti-Human/Mouse/Rat Neuropilin-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2215) 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.



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

Neuropilin-2 (Npn-2) is a 120 kDa, type I transmembrane (TM) glycoprotein that is related to the semaphorin receptor now known as Neuropilin-1 (1). Npn-2 is a complex molecule with multiple splice forms. Five transmembrane forms are known, and one 62 kDa soluble form has been identified (2). Based on the originally reported precursor size of 909 amino acids (aa), the "standard" precursor in human will have a 20 aa signal sequence, an 842 aa extracellular region, a 25 aa TM segment, and a 42 aa cytoplasmic tail (1). The extracellular region contains two N-terminal CUB (C1r/Ugef/BMP-1) domains, two jellyroll-shaped coagulation factor V type C domains, and a juxtamembrane MAM (meprin/A-5 protein/tyrosine phosphatase μ) domain (1, 3). The CUB and factor V domain are involved in VEGF and semaphorin binding. The MAM domain appears necessary for signaling through plexin-1 (4). The five transmembrane isoforms all share the same CUB, factor V and MAM domains. Splicing begins at aa 809, seven amino acids after the end of the MAM domain, and it involves the end of the extracellular region, the TM segment, and the cytoplasmic domain (a total of 101 aa). Two of the four variants show a complete replacement of these 101 aa with a totally unrelated stretch of approximately 90 aa. This creates a new TM and cytoplasmic tail. These forms are called "Npn-2b" forms. Two other isoforms (plus the standard 909 aa form) retain the 101 aa stretch, and add either 17 or 22 aa to the end of the extracellular region. These forms are called "Npn-2a" forms. The isoform offered by R&D Systems is the "a" form with the 17 aa addition. This isoform shows 94% aa identity to the equivalent regions in mouse and rat Npn-2. The soluble form of Npn-2 is 555 aa in precursor length, and contains the two CUB domains plus the first 1½ factor V type C domains (1). Npn-2 binds Sema3B through F, and VEGF isoforms 165, 145, PlGF-2, and VEGF-C (5). It is known to form homodimers and heterodimers with Npn-1, and it forms receptor complexes with plexin-1 and VEGF R1 (4, 5). Npn-2 is found on a variety of cell types including neurons (motor, autonomic, sensory), vascular endothelial cells, Schwann cells and pancreatic acinar cells.

References:

1. Chen, H. *et al.* (1997) *Neuron* **19**:547.
2. Rossignol, M. *et al.* (2000) *Genomics* **70**:211.
3. He, Z. and M. Tessier-lavigne (1997) *Cell* **90**:739.
4. Nakamura, F. and Y. Goshima (2002) *Adv. Exp. Med. Biol.* **515**:55.
5. Neufeld, G. *et al.* (2002) *Adv. Exp. Med. Biol.* **515**:81.

PRODUCT SPECIFIC NOTICES

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