

DESCRIPTION

Species Reactivity	Mouse/Rat
Specificity	Detects mouse and rat Neuropilin-2 in direct ELISAs and Western blots. In direct ELISAs, less than 10% cross-reactivity with recombinant human Neuropilin-2 and recombinant rat Neuropilin-1 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant rat Neuropilin-2 Gln23-Asp857 (Val809-Asp825 del) Accession # O35276
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.

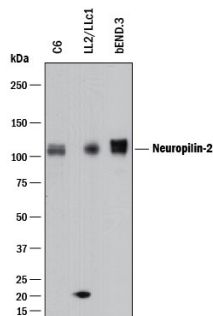
APPLICATIONS

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
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	5 µg/mL	See Below
Blockade of Receptor-ligand Interaction	In a functional ELISA, 2.5-15 µg/mL of this antibody will block 50% of the binding of 10 ng/mL of Recombinant Human VEGF ₁₆₅ (Catalog # 293-VE) to immobilized Recombinant Rat Neuropilin-2 Fc Chimera (Catalog # 567-N2) coated at 5 µg/mL (100 µL/well). At 60 µg/mL, this antibody will block >90% of the binding.	

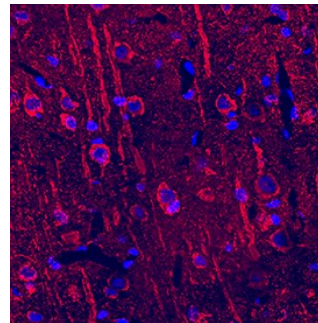
DATA

Western Blot



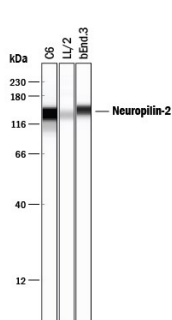
Detection of Mouse and Rat Neuropilin-2 by Western Blot. Western blot shows lysates of C6 rat glioma cell line, LL2 mouse Lewis lung carcinoma cell line, and bEnd.3 mouse endothelioma cell line. PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Mouse/Rat Neuropilin-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF567) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for Neuropilin-2 at approximately 110 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



Neuropilin-2 in Rat Brain. Neuropilin-2 was detected in perfusion fixed frozen sections of rat brain using Goat Anti-Mouse/Rat Neuropilin-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF567) at 15 µg/mL overnight at 4 °C. Tissue was 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 in neurons. View our protocol for [Fluorescent IHC Staining of Frozen Tissue Sections](#).

Simple Western



Detection of Mouse and Rat Neuropilin-2 by Simple Western™. Simple Western lane view shows lysates of C6 rat glioma cell line, LL2 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 5 µg/mL of Goat Anti-Mouse/Rat Neuropilin-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF567) 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	<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 known as Neuropilin) and Npn-2 (previously known as Npn-1-related molecule) are type I transmembrane proteins that bind members of the class III secreted semaphorin subfamily which are implicated in repulsive axon guidance. The extracellular domain of these proteins is composed of two N-terminal CUB (complement-binding) domains (domains a1 and a2), two domains with homology to coagulation factors V and VIII (domains b1 and b2) and a MAM (meprin) domain (domain c). In the absence of ligands, neuropilins can form homo- and hetero-oligomers via homophilic interactions of their MAM domains. At the amino acid sequence level, Npn-1 and Npn-2 share 44% identity. Npn-1 and Npn-2 show different binding specificities for different members of the semaphorin family. The expression patterns of Npn-1 and Npn-2 in developing neurons of the central and peripheral nervous systems are largely, though not completely nonoverlapping. Npn-1 and Npn-2 are also expressed by endothelial and tumor cells and have been shown to be isoform-specific receptors for VEGF₁₆₅. Npn-1 was also reported to bind PIGF-2 and the VEGF-like protein from of virus NZ2.

References:

1. Fujisawa, H. and T. Kitsukawa (1998) *Curr. Opin. Neurobiol.* **8**:587.
2. Neufeld, G. *et al.* (1999) *FASEB J.* **13**:9.
3. Poltorak, Z. *et al.* (2000) *J. Biol. Chem.* **275**:18040.

PRODUCT SPECIFIC NOTICES

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