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Product datasheet

Anti-Neuropilin 1 antibody [EPR3113] ab81321

敲除验证 重组 RabMAb

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概述

产品名称	Anti-Neuropilin 1抗体[EPR3113]
描述	兔单克隆抗体[EPR3113] to Neuropilin 1
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP
种属反应性	**与反应:** Mouse, Rat, Human **预测可用于:** Monkey, Common marmoset
免疫原	Synthetic peptide within Human Neuropilin 1 aa 900 to the C-terminus (intracellular). The exact sequence is proprietary. Database link: **O14786** (Peptide available as **ab189308**)
阳性对照	WB: Wild-type A549, MDA-MB-231, HUVEC and HepG2 whole cell lysate (**ab7900**), human placenta, kidney and heart, mouse heart and kidney and rat heart and kidney tissue lysates. IHC-P: Human liver tissue; Rat brain tissue; Mouse brain tissue. ICC/IF: MCF7 and HUVEC cells; Omentum and effluent-derived mesothelial cells; COS1 fibroblast-like cell line derived from monkey kidney tissue. Flow Cyt (intra): HepG2 and MCF7 cells. IHC-Fr: Human kidney tissue. IP: Mouse heart tissue lysate
常规说明	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.

存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR3113
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab81321于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/50 - 1/70. The epitope that the antibody recognizesis intracellular. Fixation and permeabilization are necessary.**ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (6)	1/1000 - 1/2000. Predicted molecular weight: 103 kDa.Can be blocked with **Neuropilin 1 peptide (ab189308)**.
IHC-P	★★★★★ (8)	1/100 - 1/400. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF	★★★★☆ (5)	1/250.
IP		1/30.

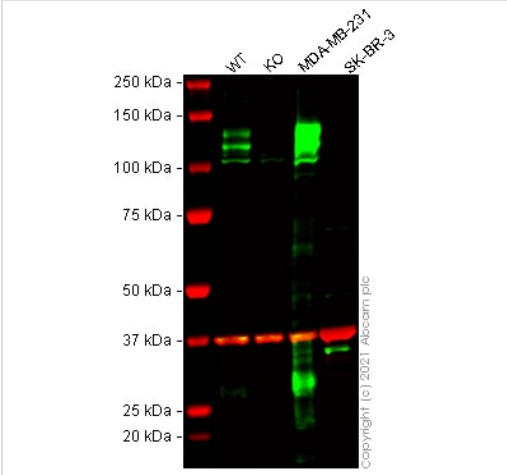
靶标

功能 The membrane-bound isoform 1 is a receptor involved in the development of the cardiovascular system, in angiogenesis, in the formation of certain neuronal circuits and in organogenesis outside the nervous system. It mediates the chemorepulsant activity of semaphorins. It binds to semaphorin 3A, The PLGF-2 isoform of PGF, The VEGF-165 isoform of VEGF and VEGF-B. Coexpression with KDR results in increased VEGF-165 binding to KDR as well as increased chemotaxis. It may regulate VEGF-induced angiogenesis.
The soluble isoform 2 binds VEGF-165 and appears to inhibit its binding to cells. It may also induce apoptosis by sequestering VEGF-165. May bind as well various members of the semaphorin family. Its expression has an averse effect on blood vessel number and integrity.

组织特异性 The expression of isoforms 1 and 2 does not seem to overlap. Isoform 1 is expressed by the blood vessels of different tissues. In the developing embryo it is found predominantly in the nervous system. In adult tissues, it is highly expressed in heart and placenta; moderately in lung, liver, skeletal muscle, kidney and pancreas; and low in adult brain. Isoform 2 is found in liver hepatocytes, kidney distal and proximal tubules.

序列相似性	Belongs to the neuropilin family. Contains 2 CUB domains. Contains 2 F5/8 type C domains. Contains 1 MAM domain.
细胞定位	Secreted and Cell membrane.

图片



Western blot - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

All lanes : Anti-Neuropilin 1 antibody [EPR3113] (ab81321) at 1/1000 dilution

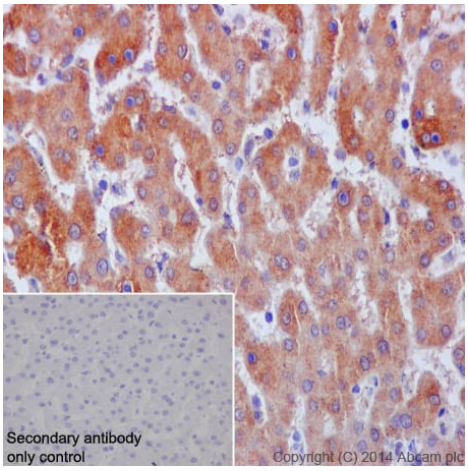
Lane 1 : Wild-type A549 cell lysate
Lane 2 : NRP1 knockout A549 cell lysate
Lane 3 : MDA-MB-231 cell lysate
Lane 4 : SK-BR-3 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

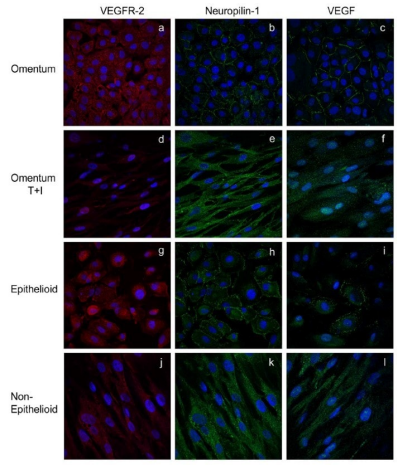
Predicted band size: 103 kDa
Observed band size: 125-135 kDa

False colour image of Western blot: Anti-Neuropilin 1 antibody [EPR3113] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab81321 was shown to bind specifically to Neuropilin 1. A band was observed at 125/135 kDa in wild-type A549 cell lysates with no signal observed at this size in NRP1 knockout cell line **ab269507** (knockout cell lysate **ab269669**). To generate this image, wild-type and NRP1 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

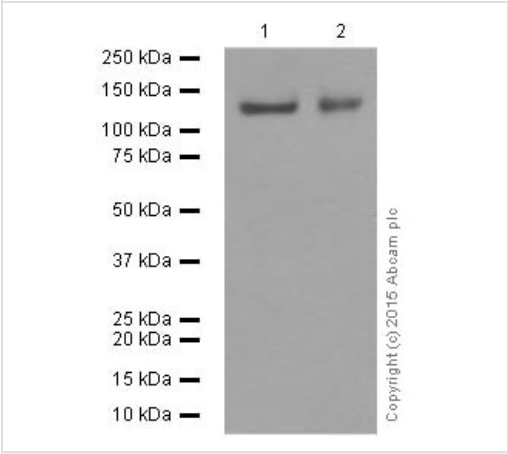
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling Neuropilin 1 with purified ab81321 at 1/400. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. **ab97051**, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunocytochemistry/ Immunofluorescence - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

Image from Pérez-Lozano ML et al., PLoS One. 2013;8(4):e60776. Fig 5.; doi: 10.1371/journal.pone.0060776.

The expression of Neuropilin 1, VEGFR-2, and VEGF was analyzed by immunofluorescence microscopy in omentum and effluent-derived mesothelial cells (MCs). MCs were double stained for Neuropilin 1 (green) and VEGFR-2 (red), and single stained for VEGF (green). Nuclei were stained with DAPI. Neuropilin 1 and VEGF show a membrane distribution in omentum and epithelioid MCs (**b, c, h, i**). During *in vitro* (**e, f**) and *ex vivo* (**k, l**) MMT both proteins change their localization and are internalized. The expression of VEGFR-2 is down-regulated but it does not show differences in localization during *in vitro* (**a, d**) and *ex vivo* (**g, j**) MMT.



Western blot - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

All lanes : Anti-Neuropilin 1 antibody [EPR3113] (ab81321) at 1/10000 dilution (purified)

Lane 1 : Mouse heart tissue lysate

Lane 2 : Rat heart tissue lysate

Lysates/proteins at 20 µg per lane.

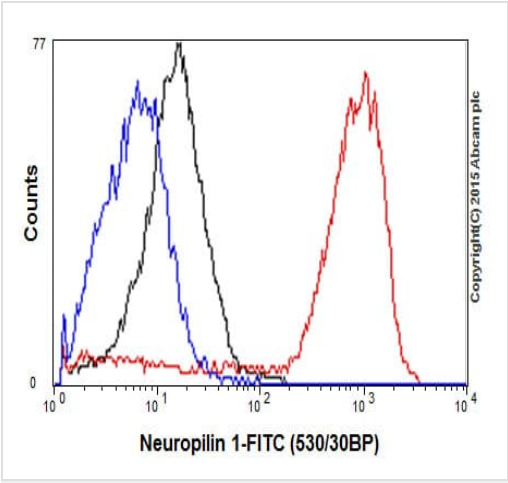
Secondary

All lanes : Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 103 kDa

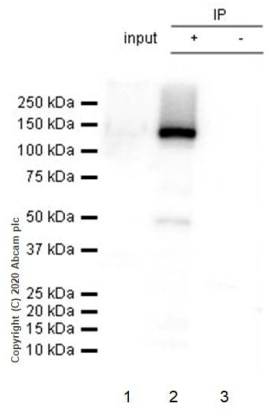
Observed band size: 120 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

Intracellular Flow Cytometry analysis of MCF7 cells labelling Neuropilin 1 with purified ab81321 at 1/70 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.

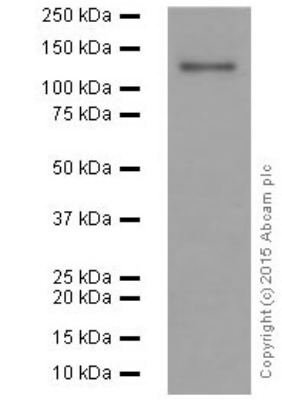


Immunoprecipitation - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

Neuropilin 1 was immunoprecipitated from 0.35mg mouse heart lysate with ab81321 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab81321 at 1/1000 dilution (0.77 µg/mL). VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used as the secondary antibody at 1/1000 dilution.

Lane 1: Mouse heart tissue lysate 10 µg
Lane 2: Mouse heart tissue lysate
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab81321 in mouse heart lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.



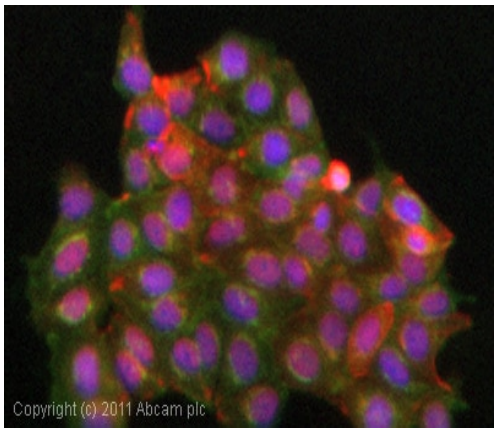
Western blot - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

Anti-Neuropilin 1 antibody [EPR3113] (ab81321) at 1/10000 dilution (purified) + Human heart tissue lysate at 20 µg

Secondary
Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

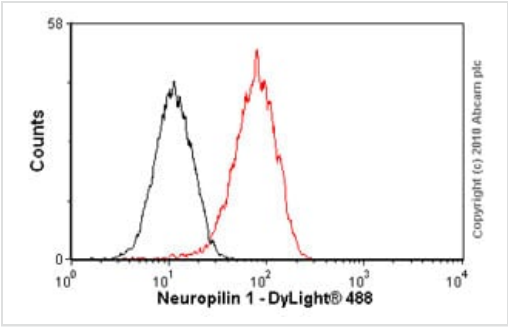
Predicted band size: 103 kDa
Observed band size: 120 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

ICC/IF image of unpurified ab81321 stained MCF7 cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (unpurified ab81321, 5µg/ml) overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti-rabbit IgG - H&L, pre-adsorbed (**ab96899**) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Flow Cytometry (Intracellular) - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

Overlay histogram showing HepG2 cells stained with unpurified ab81321 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (unpurified ab81321, 1/50 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit monoclonal IgG (0.5µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a significantly decreased signal in HepG2 cells fixed with methanol (5 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.

Western blot - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

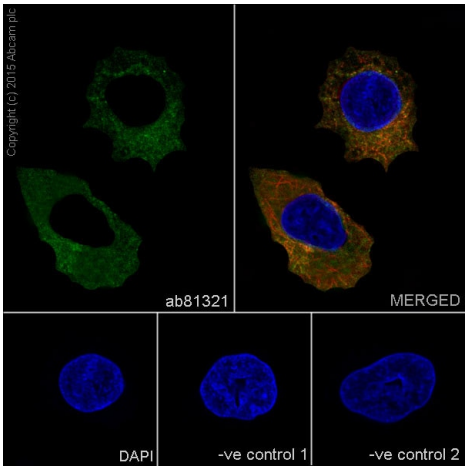
Anti-Neuropilin 1 antibody [EPR3113] (ab81321) at 1/2000 dilution (purified) + Human placenta tissue lysate at 20 µg

Secondary

Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 103 kDa
Observed band size: 120 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

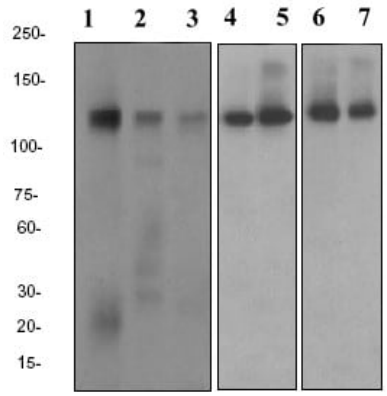


Immunocytochemistry/ Immunofluorescence - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

Immunocytochemistry/Immunofluorescence analysis of HUVEC cells labelling Neuropilin 1 with purified ab81321 at 1/250. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500) were also used.

Control 1: primary antibody (1/250) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).



Western blot - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

All lanes : Anti-Neuropilin 1 antibody [EPR3113] (ab81321) at 1/1000 dilution (unpurified)

Lane 1 : Human placenta lysate
Lane 2 : HUVEC cell lysate
Lane 3 : HepG2 cell lysate
Lane 4 : Mouse heart tissue lysate
Lane 5 : Mouse kidney tissue lysate
Lane 6 : Rat heart tissue lysate
Lane 7 : Rat kidney tissue lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

Predicted band size: 103 kDa
Observed band size: 120 kDa

Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

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