abcam

Product datasheet

Anti-Neuropilin 1 antibody [EPR3113] ab81321

Recombinant RabMAb

17 Abreviews 49 References 15 Images

Overview

Product name	Anti-Neuropilin 1 antibody [EPR3113]
Description	Rabbit monoclonal [EPR3113] to Neuropilin 1
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, IP, Flow Cyt, IHC-Fr, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human, Monkey, Common marmoset
Immunogen	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)
Positive control	WB: 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; Human peritoneal specimens; 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: HepG2 and MCF7 cells. IHC-Fr: Human kidney tissue
General notes	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. This product is a recombinant rabbit monoclonal antibody.

Properties

Form	Liquid
Storage instructions	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.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3113

Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab81321** in the following tested applications.

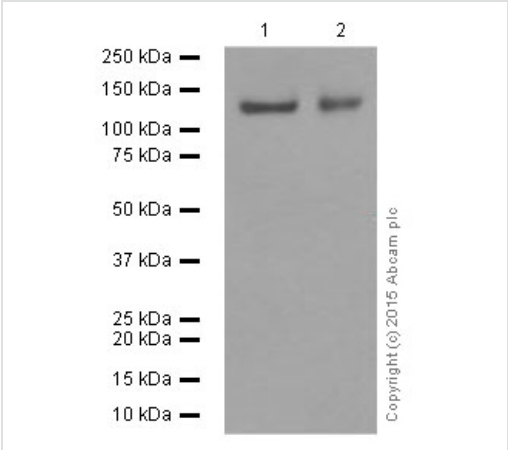
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★	1/1000 - 1/2000. Predicted molecular weight: 103 kDa.Can be blocked with Neuropilin 1 peptide (ab189308).
IHC-P	★★★★☆	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.
IP		1/100.
Flow Cyt	★★★★☆	1/50 - 1/70. The epitope that the antibody recognizes is intracellular. Fixation and permeabilization are necessary. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-Fr	★★★★☆	Use at an assay dependent concentration.
ICC/IF	★★★★☆	1/250.

Target

Function	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.
Tissue specificity	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.
Sequence similarities	Belongs to the neuropilin family. Contains 2 CUB domains. Contains 2 F5/8 type C domains. Contains 1 MAM domain.
Cellular localization	Secreted and Cell membrane.

Images



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

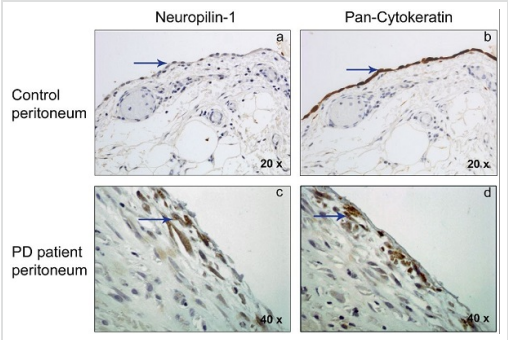
All lanes : Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 103 kDa

Observed band size: 120 kDa

why is the actual band size different from the predicted?

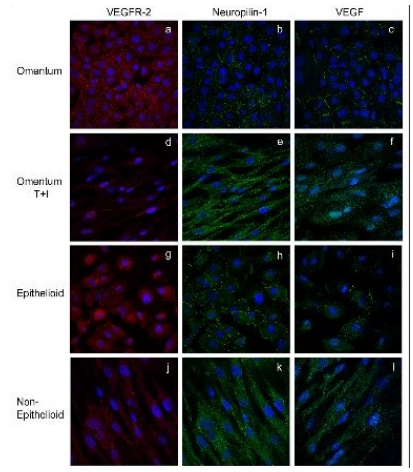
Blocking and dilution buffer: 5% NFDM/TBST.



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

Image fro Pérez-Lozano ML et al., PLoS One. 2013;8(4):e60776. Fig 6.; doi: 10.1371/journal.pone.0060776.

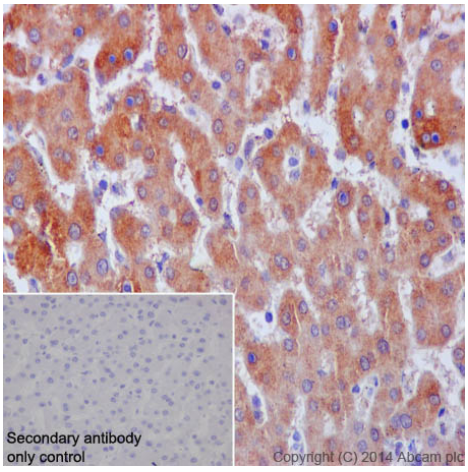
The expression of Neuropilin 1 and the mesothelial marker cytokeratin was analyzed in human peritoneal specimens by immunohistochemistry. Positive cells for antibodies used (Neuropilin 1 (ab81321) and Cytokeratin) show brown staining. Nuclei are counterstained in blue. (**a, b**) Control peritoneal tissue, with a conserved mesothelial cell monolayer showing an epithelioid morphology (with a 20X objective). These cells show weak expression of Neuropilin 1 and a marked staining for cytokeratin (arrows). No expression of these proteins was observed in the submesothelial area (region under mesothelial monolayer) (**c, d**) Fibrotic tissue sample from peritoneal dialysis (PD) patient showing the loss of mesothelial monolayer and invading spindle-like mesothelial cells in submesothelial area (with a 40X objective). These cells present a strong staining for Neuropilin 1 (**c**), and are also positive for cytokeratin (**d**) (arrows). Pictures are representative of 5 cases of PD patient samples and 4 of control samples.



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

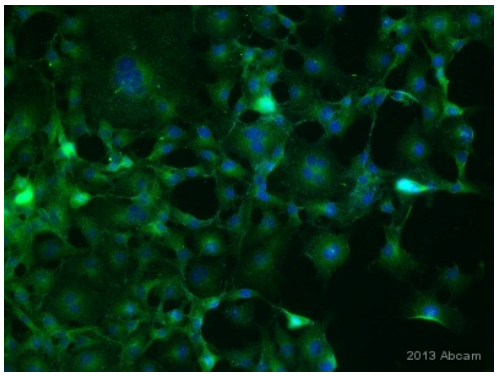
Image fro Pérez-Lozano ML et al., PLoS One. 2013;8(4):e60776. Fig 5.; doi: 10.1371/journal.pone.0060776.

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.



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

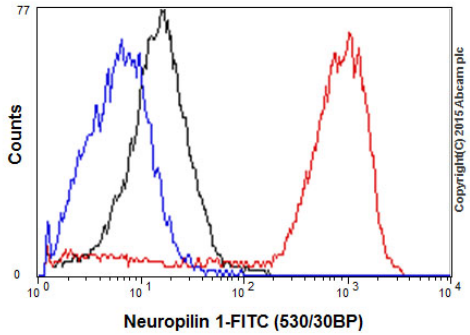
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling Neuropilin 1 with purified ab81321 at 1/400. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary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



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

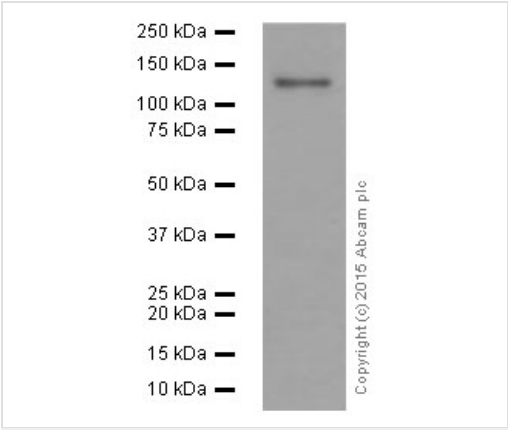
This image is courtesy of an anonymous Abreview

Unpurified ab81321 staining Neuropilin 1 in the COS1 fibroblast-like cell line derived from monkey kidney tissue by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with Triton X-100 0.1% and blocked with 10% serum for 60 minutes at 24°C. Samples were incubated with primary antibody (1/200) for 16 hours at 4°C. An Alexa Fluor® 488-conjugated goat anti-rabbit monoclonal(1/500) was used as the secondary antibody.



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

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.



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

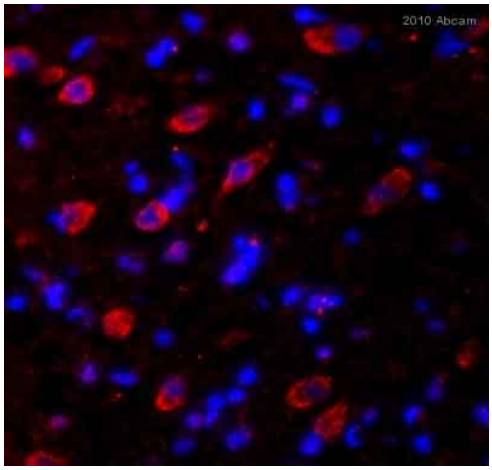
Anti-Neuropilin 1 antibody [EPR3113] (ab81321) at 1/10000 dilution (purified) + Human heart tissue lysate at 20 µg

Secondary

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

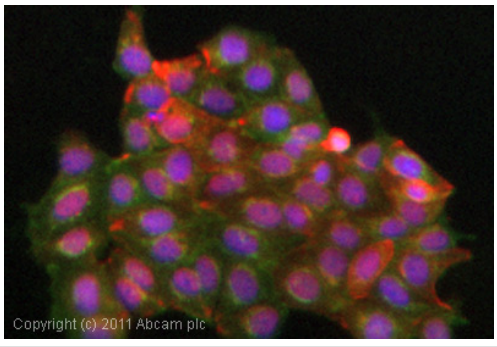
Predicted band size: 103 kDa
Observed band size: 120 kDa why is the actual band size different from the predicted?

Blocking and dilution buffer: 5% NFDM/TBST.



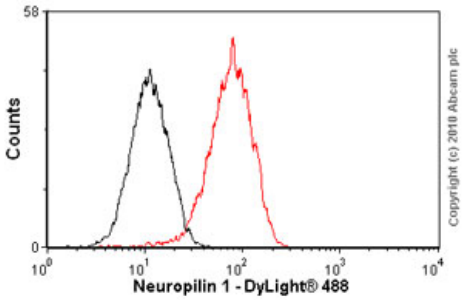
Immunohistochemistry (Frozen sections) - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)
Image courtesy of an anonymous Abreview.

Unpurified ab81321 staining Neuropilin 1 in rat brain tissue sections by Immunohistochemistry (frozen sections). Tissue was fixed with paraformaldehyde and then blocked with 10% serum for 1 hour at 27°C followed by incubation with the primary antibody, undiluted, for 14 hours at 4°C. An undiluted Cy3® conjugated donkey anti-rabbit was used as the secondary antibody.



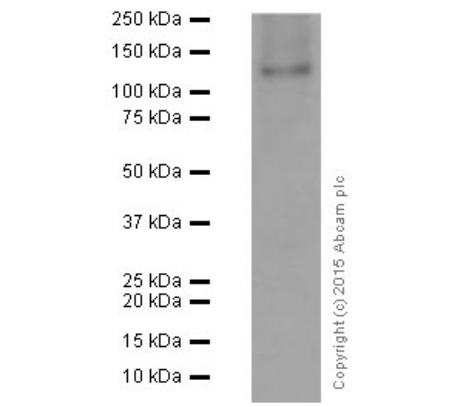
Immunocytochemistry/ Immunofluorescence - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

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 - 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)

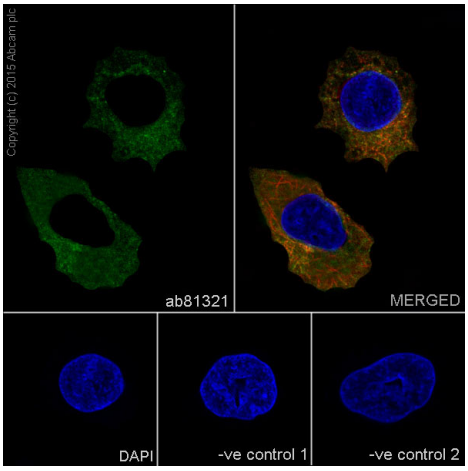
Anti-Neuropilin 1 antibody [EPR3113] (ab81321) at 1/2000 dilution (purified) + Human placenta tissue lysate at 20 µg

Secondary

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

Predicted band size: 103 kDa
Observed band size: 120 kDa why is the actual band size different from the predicted?

Blocking and dilution buffer: 5% NFDM/TBST.

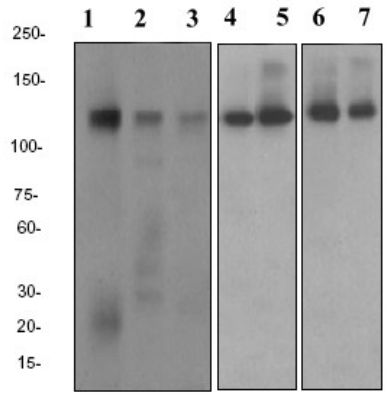


Immunocytochemistry/ Immunofluorescence - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

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)

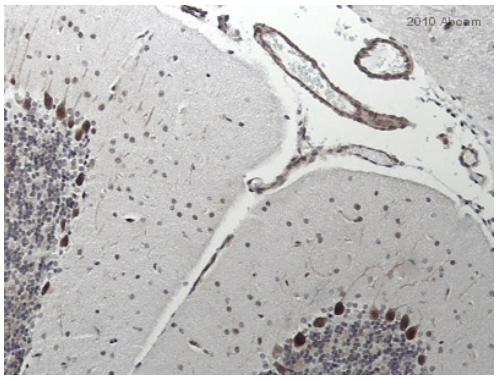
All lanes : Anti-Neuropilin 1 antibody [EPR3113] (ab81321) at 1/1000 dilution (unpurified)

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

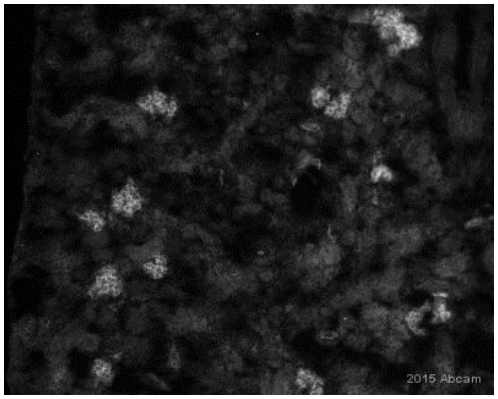
Predicted band size: 103 kDa
Observed band size: 120 kDa why is the actual band size different from the predicted?



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

This image is courtesy of an Abreview submitted by Manoj Kumar Valluru

Unpurified ab81321 staining Neuropilin 1 in mouse brain tissue sections by Immunohistochemistry (IHC-P - formaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 10% serum for 1 hour at room temperature; antigen retrieval was by heat mediation in citrate buffer (pH 6). Samples were incubated with primary antibody (1/100 in PBS + 2% blocking serum) for 16 hours at 4°C. A biotin-conjugated goat anti-rabbit IgG polyclonal (1/250) was used as the secondary antibody.



Immunohistochemistry (Frozen sections) - Anti-Neuropilin 1 antibody [EPR3113] (ab81321)

Image courtesy of an anonymous Abreview.

Immunohistochemical analysis of frozen human kidney tissue sections labelling Neurophilin 1 with ab81321 at a concentration of 1/100 for 18 hours at 4°C. The antibody was then blocked with a serum free protein block for 1 hour at 21°C. The secondary antibody used was a donkey anti-rabbit antibody conjugated to an Alexa488® dye inclubated at 1/400.

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