abcam

Product datasheet

Anti-Neuropilin 1 antibody [EPR3113] - BSA and Azide free ab184783

KO VALIDATED Recombinant RabMAb

12 Images

Overview

Product name	Anti-Neuropilin 1 antibody [EPR3113] - BSA and Azide free
Description	Rabbit monoclonal [EPR3113] to Neuropilin 1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, IP, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Monkey, Common marmoset
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	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.
General notes	ab184783 is the carrier-free version of **ab81321**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3113
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab184783 in the following tested applications.

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

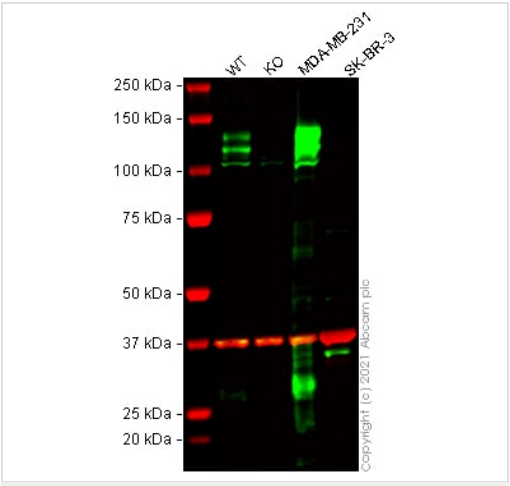
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		Use at an assay dependent concentration. Predicted molecular weight: 103 kDa. Can be blocked with Neuropilin 1 peptide (**ab189308**).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.

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] - BSA and Azide free (ab184783)

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

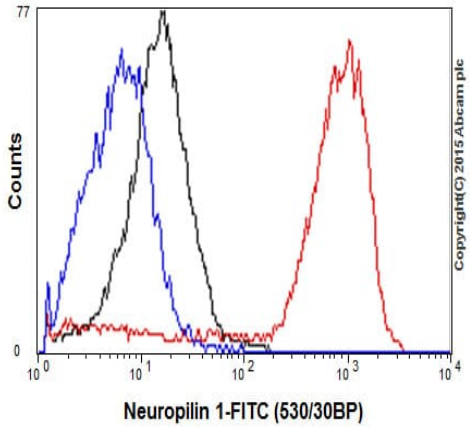
Lysates/proteins at 20 µg per lane.

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

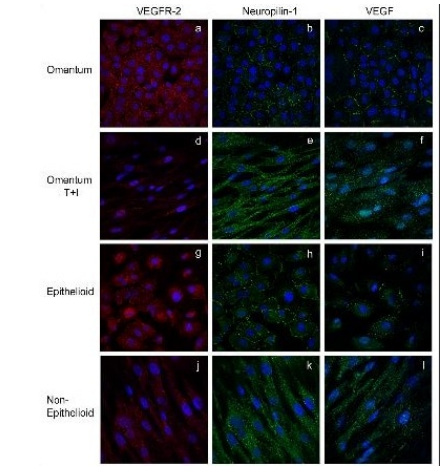
on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed 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.



Flow Cytometry (Intracellular) - Anti-Neuropilin 1 antibody [EPR3113] - BSA and Azide free (ab184783)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81321**).

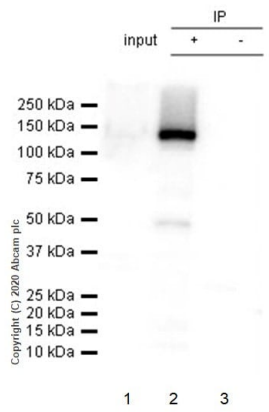


Immunocytochemistry/ Immunofluorescence - Anti-Neuropilin 1 antibody [EPR3113] - BSA and Azide free (ab184783)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81321**).



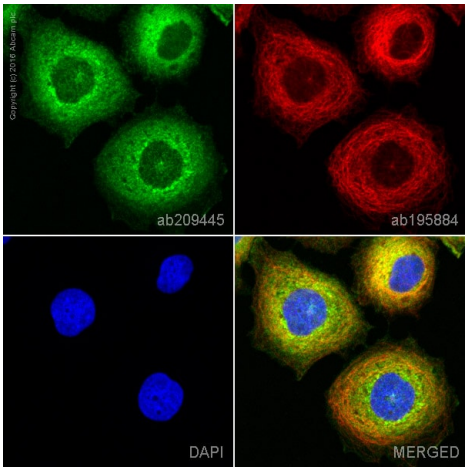
Immunoprecipitation - Anti-Neuropilin 1 antibody [EPR3113] - BSA and Azide free (ab184783)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81321**).

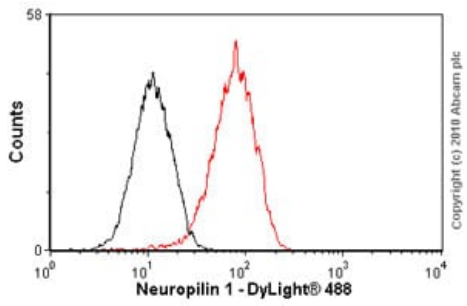


Immunocytochemistry/ Immunofluorescence - Anti-Neuropilin 1 antibody [EPR3113] - BSA and Azide free (ab184783)

Clone EPR3113 (ab184783) has been successfully conjugated by Abcam. This image was generated using Anti-Neuropilin 1 antibody [EPR3113] (PE). Please refer to **ab209445** for protocol details.

ab209445 staining Neuropilin 1 in MCF7 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab209445** at 1/100 dilution (Pseudocolored in green) and **ab195884**, Rat monoclonal to Tubulin (Alexa Fluor® 647), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

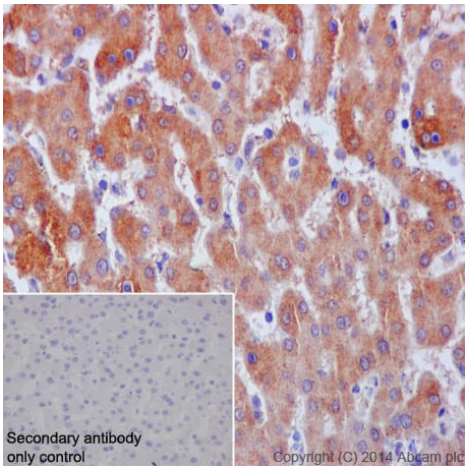


Flow Cytometry (Intracellular) - Anti-Neuropilin 1 antibody [EPR3113] - BSA and Azide free (ab184783)

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.

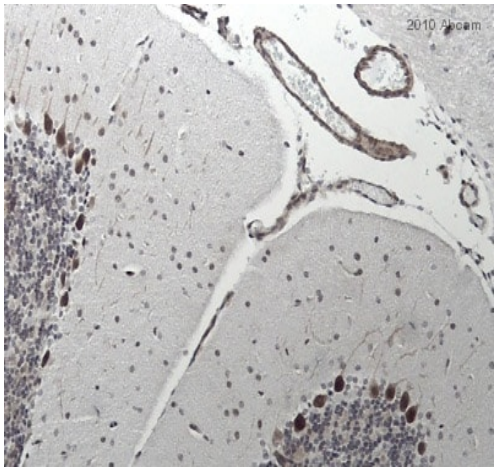
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81321**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Neuropilin 1 antibody [EPR3113] - BSA and Azide free (ab184783)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81321**).

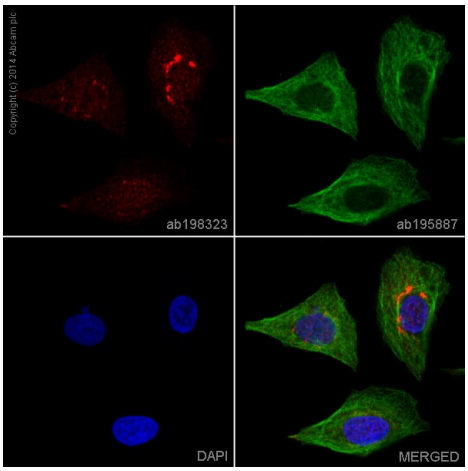


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Neuropilin 1 antibody [EPR3113] - BSA and Azide free (ab184783)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81321**).

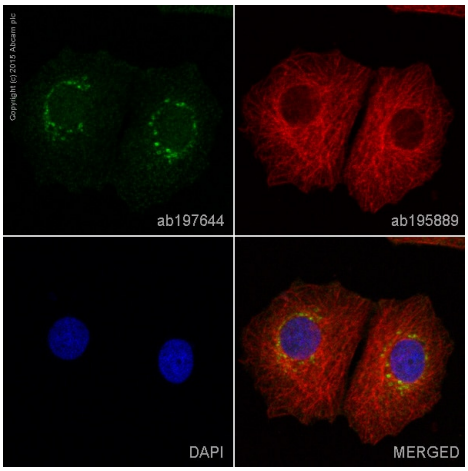


Immunocytochemistry/ Immunofluorescence - Anti-Neuropilin 1 antibody [EPR3113] - BSA and Azide free (ab184783)

Clone EPR3113 (ab184783) has been successfully conjugated by Abcam. This image was generated using Anti-Neuropilin 1 antibody [EPR3113] (Alexa Fluor® 647). Please refer to **ab198323** for protocol details.

ab198323 staining Neuropilin 1 in HeLa cells. The cells were fixed with 4% formaldehyde (10 min), permeabilised in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab198323** at 1/100 dilution (shown in red) and **ab195887**, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 488, shown in green) at 1/167 dilution overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

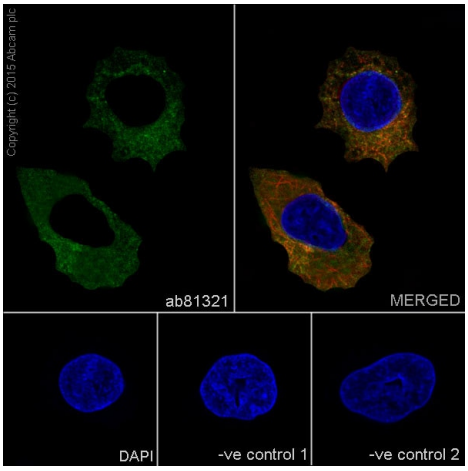


Immunocytochemistry/ Immunofluorescence - Anti-Neuropilin 1 antibody [EPR3113] - BSA and Azide free (ab184783)

Clone EPR3113 (ab184783) has been successfully conjugated by Abcam. This image was generated using Anti-Neuropilin 1 antibody [EPR3113] (Alexa Fluor® 488). Please refer to **ab197644** for protocol details.

ab197644 staining Neuropilin 1 in MCF7 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab197644** at 1/100 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



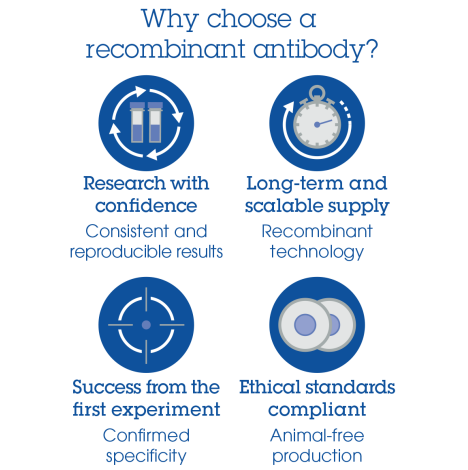
Immunocytochemistry/ Immunofluorescence - Anti-Neuropilin 1 antibody [EPR3113] - BSA and Azide free (ab184783)

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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab81321**).



Anti-Neuropilin 1 antibody [EPR3113] - BSA and Azide free (ab184783)

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