Anti-Neuropilin-1 Antibody [ST05-30]

ET1609-69

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP
Molecular Wt:	Predicted band size: 103 kDa
Clone number:	ST05-30

Description: Neuropilin is a type I transmembrane receptor that has been implicated in aspects of axon growth and guidance and has been shown to act as a high affinity receptor for class III semaphorins and vascular endothelial growth factor (VEGF). A closely related protein, neuropilin-2, shares a common domain structure and signifcant homology with neuropilin and also acts as a receptor for the class III semaphorins and VEGF. Both neuropilins are involved in regulating many physiological pathways including axonal guidance and angiogenesis, however they exhibit differential expression in the adult vasculature. Neuropilin-2 is polysialylated and expressed on the surface of dendritic cells. It is also expressed by venous and lymphatic endothelium. Neuropilin is expressed predominantly by arterial endothelium.

Immunogen: Synthetic peptide within Human Neuropilin-1 aa 881-923 / 923.

Positive control: U-87 MG cell lysate, A549 cell lysate, MDA-MB-231 cell lysate, mouse brain tissue lysate, rat brain tissue lysate, human liver tissue, mouse heart tissue lysate, rat heart tissue lysate, SHG-44, MCF-7, HUVEC, human kidney tissue, mouse kidney tissue.

Subcellular location: Cell membrane, Secreted.

Database links: SwissProt: O14786 Human | P97333 Mouse | Q9QWJ9 Rat

Recommended Dilutions:

WB	1:2,000-1:5,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:200
IP	Use at an assay dependent concentration.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

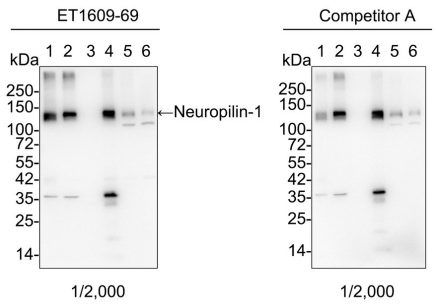

Fig1: Western blot analysis of Neuropilin-1 on different lysates with Rabbit anti-Neuropilin-1 antibody (ET1609-69) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: U-87 MG cell lysate
Lane 2: A549 cell lysate
Lane 3: SK-Br-3 cell lysate (low expression)
Lane 4: MDA-MB-231 cell lysate
Lane 5: Mouse brain tissue lysate
Lane 6: Rat brain tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 103 kDa
Observed band size: 130 kDa

Exposure time: 35 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1609-69) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

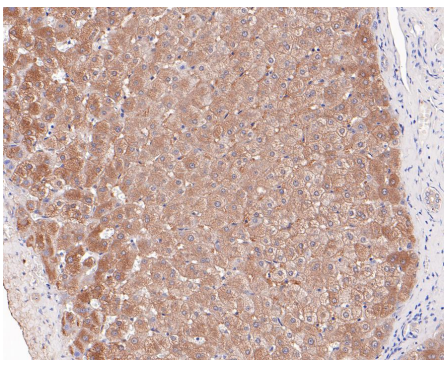

Fig2: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-Neuropilin-1 antibody (ET1609-69) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-69) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

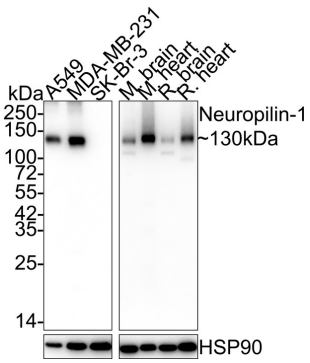

Fig3: Western blot analysis of Neuropilin-1 on different lysates with Rabbit anti-Neuropilin-1 antibody (ET1609-69) at 1/5,000 dilution.

Lane 1: A549 cell lysate
Lane 2: MDA-MB-231 cell lysate
Lane 3: SK-Br-3 cell lysate (low expression)
Lane 4: Mouse brain tissue lysate
Lane 5: Mouse heart tissue lysate
Lane 6: Rat brain tissue lysate
Lane 7: Rat heart tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 103 kDa
Observed band size: 130 kDa

Exposure time: Lane 1-3: 1 minute 21 seconds; Lane 4-7: 43 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1609-69) at 1/5,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

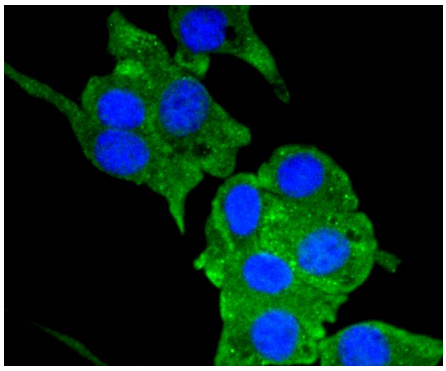

Fig4: ICC staining of Neuropilin-1 in SHG-44 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1609-69, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

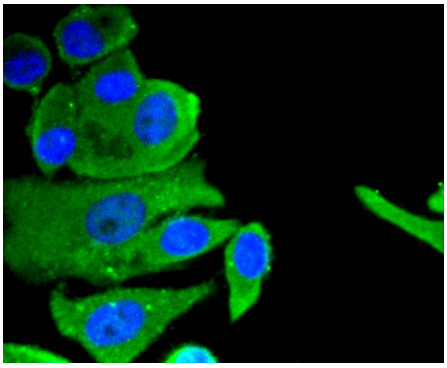

Fig5: ICC staining of Neuropilin-1 in MCF-7 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1609-69, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

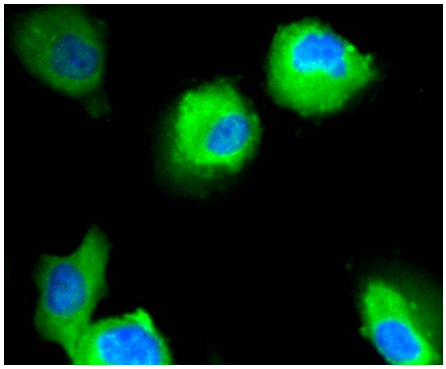

Fig6: ICC staining of Neuropilin-1 in HUVEC cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1609-69, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

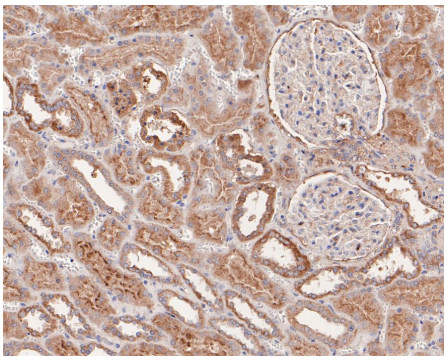

Fig7: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Neuropilin-1 antibody (ET1609-69) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-69) at 1/50 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

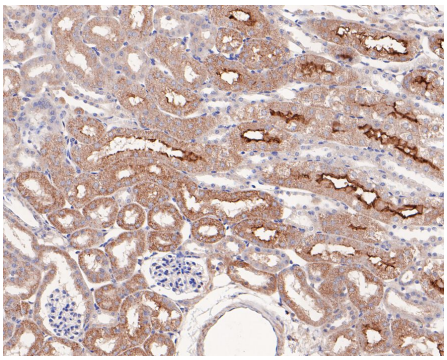

Fig8: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Neuropilin-1 antibody (ET1609-69) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1609-69) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

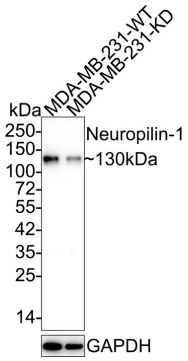

Fig9: Western blot analysis of Neuropilin-1 on different lysates with Rabbit anti-Neuropilin-1 antibody (ET1609-69) at 1/5,000 dilution.

Lane 1: MDA-MB-231-si NT cell lysate
Lane 2: MDA-MB-231-si Neuropilin-1 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 103 kDa
Observed band size: 130 kDa

Exposure time: 1 minute 2 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1609-69) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Background References

1. Liu F et al. Prostate cancer cells induce osteoblastic differentiation via semaphorin 3A. Prostate 75:370-80 (2015).
2. Linthicum FH et al. The periductal channels of the endolymphatic duct, hydrodynamic implications. Otolaryngol Head Neck Surg 150:441-7 (2014).