abcam

Product datasheet

Anti-NF2 / Merlin antibody [AF1G4] ab88957

KO VALIDATED

11 References 5 Images

Overview

Product name	Anti-NF2 / Merlin antibody [AF1G4]
Description	Mouse monoclonal [AF1G4] to NF2 / Merlin
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, WB, IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant human NF2/ Merlin with His tag purified from E.coli
Positive control	HeLa, 293T, MCF7, HepG2, MOLT4, C6 and L929 cell lysates. Human skeletal muscle FFPE tissue sections
General notes	This product was changed from ascites to tissue culture supernatant on 18th September 2017. Lot number GR310755 and higher lots are from tissue culture supernatant. Please note that the dilutions may need to be adjusted accordingly. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid repeated freeze / thaw cycles.
Storage buffer	Preservative: 0.03% Sodium azide Constituents: HEPES, 0.01% BSA, 50% Glycerol, 0.87% Sodium chloride
Purity	Tissue culture supernatant
Clonality	Monoclonal
Clone number	AF1G4
Isotype	IgG2a

Light chain type kappa

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab88957 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
ICC/IF		Use at an assay dependent concentration.
WB		Use a concentration of 0.5 µg/ml. Detects a band of approximately 69 kDa (predicted molecular weight: 69 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Target

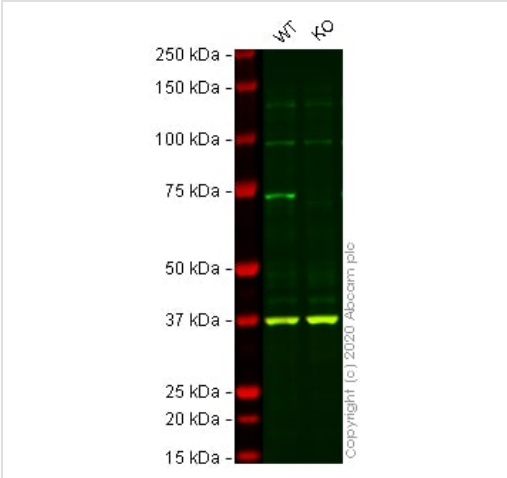
Function Probable regulator of the Hippo/SWH (Sav/Wts/Hpo) signaling pathway, a signaling pathway that plays a pivotal role in tumor suppression by restricting proliferation and promoting apoptosis. Along with WWC1 can synergistically induce the phosphorylation of LATS1 and LATS2 and can probably function in the regulation of the Hippo/SWH (Sav/Wts/Hpo) signaling pathway. May act as a membrane stabilizing protein. May inhibit PI3 kinase by binding to AGAP2 and impairing its stimulating activity. Suppresses cell proliferation and tumorigenesis by inhibiting the CUL4A-RBX1-DDB1-VprBP/DCAF1 E3 ubiquitin-protein ligase complex.

Tissue specificity Widely expressed. Isoform 1 and isoform 3 are predominant. Isoform 4, isoform 5 and isoform 6 are expressed moderately. Isoform 8 is found at low frequency. Isoform 7, isoform 9 and isoform 10 are not expressed in adult tissues, with the exception of adult retina expressing isoform 10. Isoform 9 is faintly expressed in fetal brain, heart, lung, skeletal muscle and spleen. Fetal thymus expresses isoforms 1, 7, 9 and 10 at similar levels.

Involvement in disease Defects in NF2 are the cause of neurofibromatosis 2 (NF2) [MIM:101000]; also known as central neurofibromatosis. NF2 is a genetic disorder characterized by bilateral vestibular schwannomas (formerly called acoustic neuromas), schwannomas of other cranial and peripheral nerves, meningiomas, and ependymomas. It is inherited in an autosomal dominant fashion with full penetrance. Affected individuals generally develop symptoms of eighth-nerve dysfunction in early adulthood, including deafness and balance disorder. Although the tumors of NF2 are histologically benign, their anatomic location makes management difficult, and patients suffer great morbidity and mortality.
Defects in NF2 are a cause of schwannomatosis (SCHWA) [MIM:162091]; also known as congenital cutaneous neurilemmomatosis. Schwannomas are benign tumors of the peripheral nerve sheath that usually occur singly in otherwise normal individuals. Multiple schwannomas in the same individual suggest an underlying tumor-predisposition syndrome. The most common such syndrome is NF2. The hallmark of NF2 is the development of bilateral vestibular-nerve schwannomas; but two-thirds or more of all NF2-affected individuals develop schwannomas in other locations, and dermal schwannomas may precede vestibular tumors in NF2-affected children. There have been several reports of individuals with multiple schwannomas who do not show evidence of vestibular schwannoma. Clinical report suggests that schwannomatosis is a

	clinical entity distinct from other forms of neurofibromatosis.
Sequence similarities	Contains 1 FERM domain.
Post-translational modifications	Phosphorylation of Ser-518 inhibits nuclear localization by disrupting the intramolecular association of the FERM domain with the C-terminal tail. Ubiquitinated by the CUL4A-RBX1-DDB1-DCAF1/VprBP E3 ubiquitin-protein ligase complex for ubiquitination and subsequent proteasome-dependent degradation.
Cellular localization	Cytoplasm > perinuclear region. Cytoplasmic granule. Observed in cytoplasmic granules concentrated in a perinuclear location. Isoform 7 is absent from ruffling membranes and filopodia; Cytoplasm > perinuclear region. Cytoplasmic granule. Observed in cytoplasmic granules concentrated in a perinuclear location. Isoform 9 is absent from ruffling membranes and filopodia; Nucleus. Cell projection > filopodium membrane. Cell projection > ruffle membrane. Cytoplasm > perinuclear region. Cytoplasmic granule. Cytoplasm > cytoskeleton. In a fibroblastic cell line, isoform 10 is found homogeneously distributed over the entire cell, with a particularly strong staining in ruffling membranes and filopodia and Cell projection > filopodium membrane. Cell projection > ruffle membrane. Nucleus. In a fibroblastic cell line, isoform 1 is found homogeneously distributed over the entire cell, with a particularly strong staining in ruffling membranes and filopodia. Colocalizes with MPP1 in non-myelin-forming Schwann cells. Binds with VPRBP in the nucleus. The intramolecular association of the FERM domain with the C-terminal tail promotes nuclear accumulation. The unphosphorylated form accumulates predominantly in the nucleus while the phosphorylated form is largely confined to the non-nuclear fractions.

Images



Western blot - Anti-NF2 / Merlin antibody [AF1G4] (ab88957)

All lanes : Anti-NF2 / Merlin antibody [AF1G4] (ab88957) at 1/2000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : NF2 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

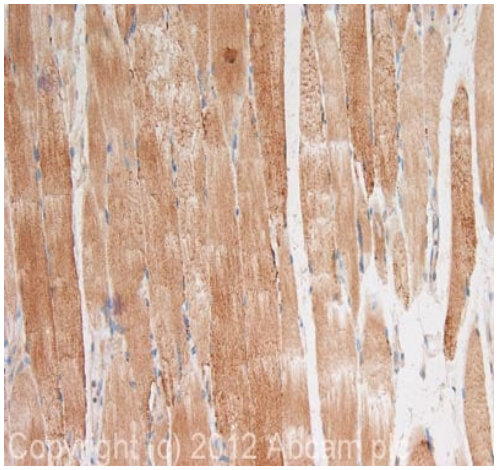
Performed under reducing conditions.

Predicted band size: 69 kDa
Observed band size: 60 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab88957 observed at 60 kDa. Red - loading control ab181602 (Rabbit Anti-GAPDH antibody [EPR16891]) observed at 37kDa.

ab88957 was shown to react with NF2 in wild-type HeLa cells in western blot with loss of signal observed in NF2 knockout cell line ab261796 (NF2 knockout cell lysate ab257179). Wild-type and NF2 knockout HeLa cell lysates were subjected to SDS-PAGE.

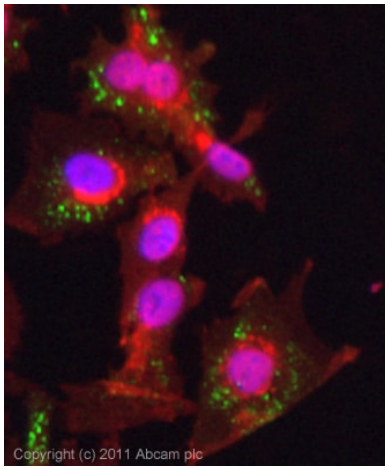
Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab88957 and ab181602 (Rabbit Anti-GAPDH antibody [EPR16891]) overnight at 4°C at a 1 in 2000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (ab216772) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (ab216777) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NF2 / Merlin antibody [AF1G4] (ab88957)

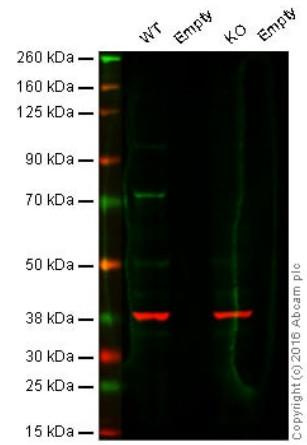
IHC image of NF2/Merlin staining in human skeletal muscle formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab88957, 10µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunocytochemistry/ Immunofluorescence - Anti-NF2 / Merlin antibody [AF1G4] (ab88957)

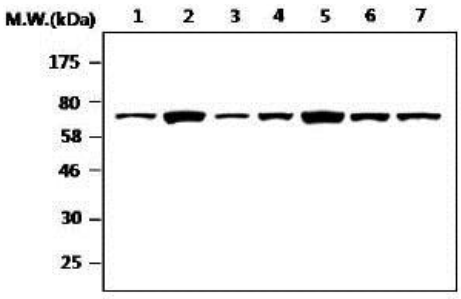
ICC/IF image of ab88957 stained HeLa cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab88957, 1/200 dilution) overnight at +4°C. The secondary antibody (green) was ab88957, DyLight® 488 goat anti-mouse IgG (H+L) 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.



Western blot - Anti-NF2 / Merlin antibody [AF1G4] (ab88957)

Lane 1: Wild-type HAP1 cell lysate (40 µg)
Lane 2: Empty lane
Lane 3: NF2 / Merlin knockout HAP1 cell lysate (20 µg)
Lane 4: Empty lane
Lanes 1 - 4: Merged signal (red and green). Green - ab88957 observed at 70 kDa. Red - loading control, ab181602, observed at 37 kDa.

ab88957 was shown to specifically react with NF2/Merlin in wild-type HAP1 cells. No band was observed when NF2 / Merlin knockout samples were examined. Wild-type and NF2/Merlin knockout samples were subjected to SDS-PAGE. Ab88957 and ab181602 (loading control to GAPDH) were diluted at 1/500 and 1/10,000 dilution respectively and incubated overnight at 4^{o}C. Blots were developed with IRDye® 800CW Goat anti-Mouse IgG (H + L) ab216772 and IRDye® 680 Goat anti-Rabbit IgG (H + L) ab216777 secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-NF2 / Merlin antibody [AF1G4] (ab88957)

All lanes : Anti-NF2 / Merlin antibody [AF1G4] (ab88957) at 1/2000 dilution

Lane 1 : HeLa cell lysate
Lane 2 : 293T cell lysate
Lane 3 : MCF7 cell lysate
Lane 4 : HepG2 cell lysate
Lane 5 : MOLT4 cell lysate
Lane 6 : C6 cell lysate
Lane 7 : L929 cell lysate

Predicted band size: 69 kDa
Observed band size: 69 kDa

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