abcam

ab281830

Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR23510-76
Purification technique	Affinity purification Protein A
Concentration	0.991 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

Flow Cyt (Intra)

Tested

Species	Mouse, Rat, Human
Dilution info	-

Notes	-

Not recommended

Species	Synthetic peptide - Mouse
Dilution info	-
Notes	-

ELISA

Tested

Species	Synthetic peptide - Mouse
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat, Human
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Mouse
Dilution info	-
Notes	-

IHC-Fr

Tested

Species	Mouse
Dilution info	-
Notes	Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20)

Species	Rat
Dilution info	-
Notes	Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20)

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide - Mouse
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Mouse
Dilution info	-

Notes	-

IP

Tested

Species	Mouse, Rat
Dilution info	-
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide - Mouse
Dilution info	-
Notes	-

IHC-P

Tested

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Mouse
Dilution info	-
Notes	-

Target data

See full target information NEFM

Function	Neurofilaments usually contain three intermediate filament proteins: NEFL, NEFM, and NEFH which are involved in the maintenance of neuronal caliber. May additionally cooperate with the neuronal intermediate filament proteins PRPH and INA to form neuronal filamentous networks (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C

Notes

ab281830 is the carrier-free version of ab254348.

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility

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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The 160 kD Neurofilament Medium also known as NEFM NF-M or NEF-M belongs to the family of neurofilament proteins. This protein is an important structural component of neurons particularly in the axonal cytoskeleton. Neurofilament Medium has a molecular mass of around 160 kilodaltons (kD). It shows high levels of expression in neuronal tissues contributing to the maintenance of axonal caliber. Commonly research recognizes it alongside other neurofilament proteins emphasizing its role in providing structural integrity to neurons.
Biological function summary	Neurofilament Medium participates in the formation of a stable network of neurofilaments within neurons. Acting as a part of the intermediate filament protein family it forms a complex with other neurofilaments such as NF-L and NF-H. This complex supports neuron structure and plays an important role in axonal transport. The heteropolymeric nature of neurofilaments contributes to their mechanical stability facilitating essential neuronal functions by aligning neurofilaments along the axon.
Pathways	Neurofilament Medium integrates into the cytoskeletal arrangement pathways that govern axonal transport and stability. It plays a significant role in the neuronal transport pathway associating with other proteins such as dynein and kinesin responsible for the motor functions along axons. Also it relates to the MAP kinase pathway where phosphorylation events modulate its assembly and disassembly dynamics in response to cellular signals.
Associated diseases and disorders	Neurofilament Medium closely relates to neurodegenerative conditions such as amyotrophic lateral sclerosis (ALS) and Alzheimer's disease. Aberrant phosphorylation or aggregation of NEFM can disrupt neuronal function leading to pathology. In ALS the abnormal accumulation of neurofilaments correlates with motor neuron degeneration. In Alzheimer's its interaction with tau protein poses significant interest as altered states of either could exacerbate neurofibrillary tangles worsening cognitive decline.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

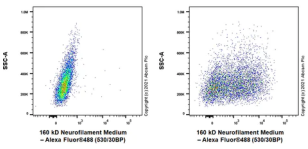
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

21 product images

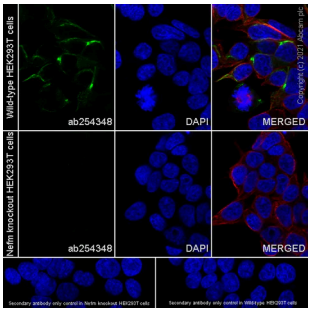


Flow Cytometry (Intracellular) - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

160 kD Neurofilament Medium Flow Cytometry (Intracellular) staining using rabbit Anti-160 kD Neurofilament Medium antibody

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized wild-type HEK-293T (human embryonic kidney epithelial cell, Right)/ 160 kD Neurofilament Medium knockout HEK-293T cells (Left) labelling 160 kD Neurofilament Medium with ab254348 at 1/500 dilution (0.1 µg). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.

Positive staining on human wild-type HEK-293T cell line (ab255449), while no staining on human NEFM knockout HEK-293T cells (ab266741).



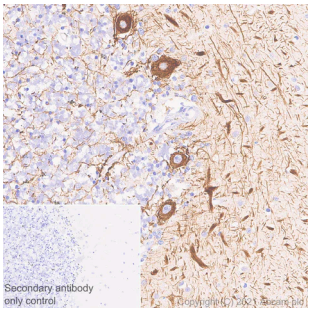
Immunocytochemistry/ Immunofluorescence - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

160 kD Neurofilament Medium Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-160 kD Neurofilament Medium antibody

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
Immunofluorescent analysis of parental HEK-293T (EDWT04) and NEFM KO HEK-293T (ED040746) cells labelling 160 kD Neurofilament Medium with ab254348 at 1/50 (8.86 µg/ml) dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was (Blue).

Confocal image showing cytoplasmic staining in parental HEK-293T cells and no staining in NEFM KO HEK-293T cells.

Secondary antibody only control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

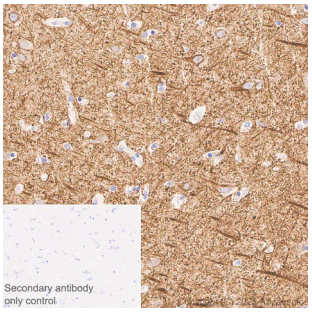
160 kD Neurofilament Medium Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-160 kD Neurofilament Medium antibody

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded human cerebellum tissue labelling 160 kD Neurofilament Medium with ab254348 at 1/4000 (0.111 µg/ml) dilution followed by ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). The section was incubated with ab254348 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Positive staining on human cerebellum.

Secondary antibody only control: Secondary antibody is ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

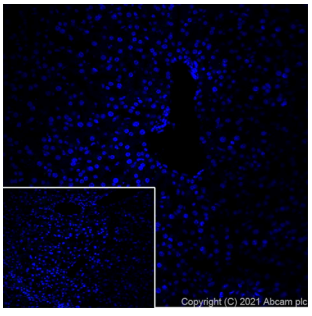
160 kD Neurofilament Medium Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-160 kD Neurofilament Medium antibody

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded human cerebrum tissue labelling 160 kD Neurofilament Medium with ab254348 at 1/4000 (0.111 µg/ml) dilution followed by ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). The section was incubated with ab254348 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Positive staining on human cerebrum.

Secondary antibody only control: Secondary antibody is ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunohistochemistry (Frozen sections) - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

160 kD Neurofilament Medium Immunohistochemistry (Frozen sections) staining using rabbit Anti-160 kD Neurofilament Medium antibody

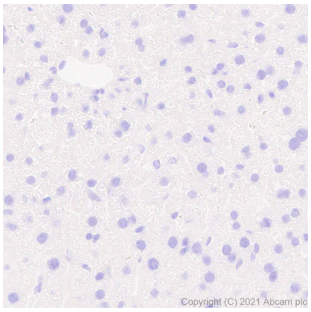
This data was developed using ab254348, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen mouse liver tissue labeling 160 kD Neurofilament Medium with ab254348 at 1/100 (4.43 µg/ml) dilution followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (Green). The nuclear counterstain was DAPI (blue).

Negative control: No staining on mouse liver (PMID: 30541916) is observed.

Secondary antibody control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.

Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

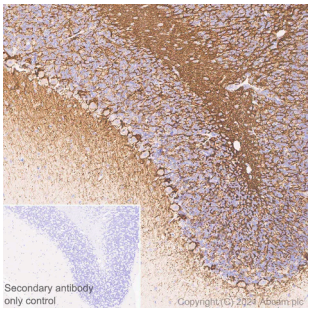
160 kD Neurofilament Medium Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-160 kD Neurofilament Medium antibody

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded mouse liver tissue labelling 160 kD Neurofilament Medium with ab254348 at 1/4000 (0.111 µg/ml) dilution followed by ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). The section was incubated with ab254348 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Negative control: No staining on mouse liver.

Secondary antibody only control: Secondary antibody is ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

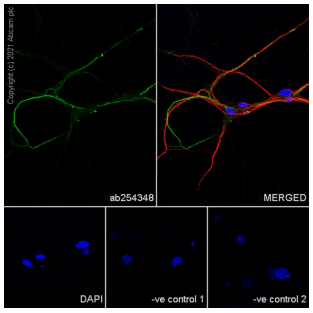
160 kD Neurofilament Medium Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-160 kD Neurofilament Medium antibody

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue labelling 160 kD Neurofilament Medium with ab254348 at 1/4000 (0.111 µg/ml) dilution followed by ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). The section was incubated with ab254348 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Positive staining on mouse cerebellum.

Secondary antibody only control: Secondary antibody is ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

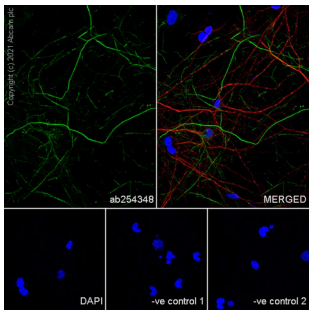
160 kD Neurofilament Medium Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-160 kD Neurofilament Medium antibody

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
Immunofluorescent analysis of mouse primary neural/glia cells fixed with 4% Paraformaldehyde and permeabilised with 0.1% TritonX-100, labelling 160 kD Neurofilament Medium with ab254348 at 1/50 (8.86 µg/ml) dilution. ab11267 Anti-MAP2 mouse monoclonal antibody was used to counterstain at 1/500 (4 µg/ml). Followed by secondary ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 (2 µg/ml) (Green). ab150120 Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) was used as secondary counterstain at 1/1000 (2 µg/ml) (Red). The nuclear counterstain was DAPI (Blue).

Confocal image showing cytoplasmic staining in mouse primary neuron cells.
Confocal scanning Z step was set as 0.3 µm followed by image processing with maximum Z projection.

Negative Control 1: ab150120 1/1000 (2 µg/ml)

Negative Control 2: ab11267 1/500 (4 µg/ml), secondary: ab150077 1/1000 (2µg/ml)



Immunocytochemistry/ Immunofluorescence - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

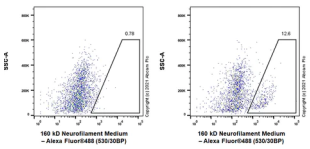
160 kD Neurofilament Medium Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-160 kD Neurofilament Medium antibody

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
Immunofluorescent analysis of rat primary neural/glia cells fixed with 4% Paraformaldehyde and permeabilised with 0.1% TritonX-100, labelling 160 kD Neurofilament Medium with ab254348 at 1/50 (8.86 µg/ml) dilution. ab11267 Anti-MAP2 mouse monoclonal antibody was used to counterstain at 1/500 (4 µg/ml). Followed by secondary ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 (2 µg/ml) (Green). ab150120 Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) was used as secondary counterstain at 1/1000 (2 µg/ml) (Red). The nuclear counterstain was DAPI (Blue).

Confocal image showing cytoplasmic staining in rat primary neuron cells.
Confocal scanning Z step was set as 0.3 µm followed by image processing with maximum Z projection.

Negative Control 1: ab150120 1/1000 (2 µg/ml)

Negative Control 2: ab11267 1/500 (4 µg/ml), secondary: ab150077 1/1000 (2 µg/ml)

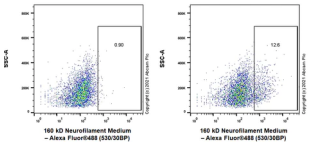


Flow Cytometry (Intracellular) - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

160 kD Neurofilament Medium Flow Cytometry (Intracellular) staining using rabbit Anti-160 kD Neurofilament Medium antibody

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
Flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized rat primary neuron cells cells labelling 160 kD Neurofilament Medium with ab254348 at 1/500 dilution (0.1 µg)/ Right compared with a Rabbit monoclonal IgG isotype control (ab172730) / Left.

A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.

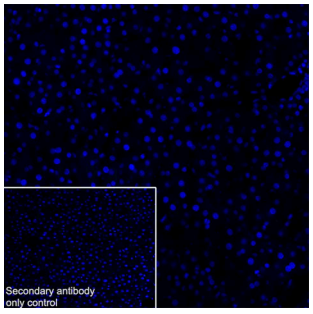


Flow Cytometry (Intracellular) - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

160 kD Neurofilament Medium Flow Cytometry (Intracellular) staining using rabbit Anti-160 kD Neurofilament Medium antibody

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Flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized mouse primary neuron cells labelling 160 kD Neurofilament Medium with ab254348 at 1/500 dilution (0.1 µg)/ Right compared with a Rabbit monoclonal IgG isotype control (ab172730) / Left.

A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



Immunohistochemistry (Frozen sections) - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

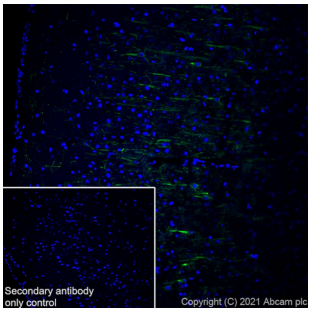
160 kD Neurofilament Medium Immunohistochemistry (Frozen sections) staining using rabbit Anti-160 kD Neurofilament Medium antibody

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen rat liver tissue labeling 160 kD Neurofilament Medium with ab254348 at 1/100 (4.43 µg/ml) dilution followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (Green). The nuclear counterstain was DAPI (blue).

Negative control: No staining on rat liver (PMID: 30541916) is observed.

Secondary antibody control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution.

Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).



Immunohistochemistry (Frozen sections) - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

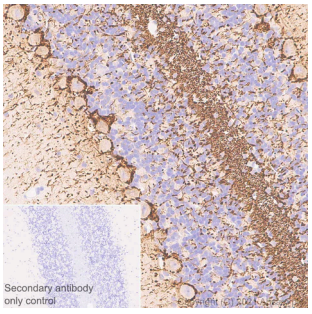
160 kD Neurofilament Medium Immunohistochemistry (Frozen sections) staining using rabbit Anti-160 kD Neurofilament Medium antibody

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen rat cerebrum tissue labeling 160 kD Neurofilament Medium with ab254348 at 1/100 (4.43 µg/ml) dilution followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (Green). The nuclear counterstain was DAPI (Blue).

Positive staining on rat cerebrum is observed.

Secondary antibody control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488)at 1/1000 dilution.

Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

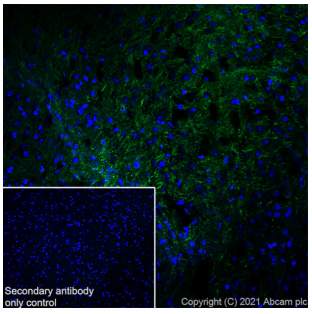
160 kD Neurofilament Medium Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-160 kD Neurofilament Medium antibody

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue labelling 160 kD Neurofilament Medium with ab254348 at 1/4000 (0.111 µg/ml) dilution followed by ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). The section was incubated with ab254348 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Positive staining on rat cerebellum.

Secondary antibody only control: Secondary antibody is ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunohistochemistry (Frozen sections) - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

160 kD Neurofilament Medium Immunohistochemistry (Frozen sections) staining using rabbit Anti-160 kD Neurofilament Medium antibody

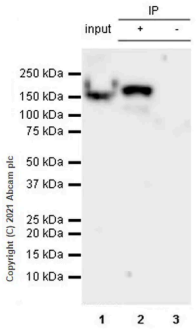
This data was developed using ab254348, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen mouse cerebrum tissue labeling 160 kD Neurofilament Medium with ab254348 at 1/100 (4.43 µg/ml) dilution followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (Green). The nuclear counterstain was DAPI (Blue).

Positive staining on mouse cerebrum is observed.

Secondary antibody control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488)at 1/1000 dilution.

Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).



Immunoprecipitation - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
160 kD Neurofilament Medium was immunoprecipitated from 0.35 mg rat brain tissue lysate 10 µg with ab254348 at 1/30 dilution (2 µg in 0.35 mg lysates). Western blot was performed on the immunoprecipitate using ab254348 at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/5000 dilution.

Lane 1: Rat brain tissue lysate 10 µg.

Lane 2: ab254348 IP in rat brain tissue lysate.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab254348 in rat brain tissue lysate.

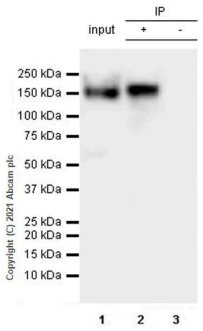
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 5.5 secs.

All lanes:
Immunoprecipitation - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - Neuronal Marker (ab254348)

Predicted band size: 102 kDa

Observed band size: 160 kDa



Immunoprecipitation - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
160 kD Neurofilament Medium was immunoprecipitated from 0.35 mg mouse brain tissue lysate 10 µg with ab254348 at 1/30 dilution (2 µg in 0.35 mg lysates). Western blot was performed on the immunoprecipitate using ab254348 at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/5000 dilution.

Lane 1: Mouse brain tissue lysate 10 µg.

Lane 2: ab254348 IP in mouse brain tissue lysate.

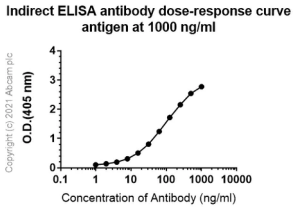
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab254348 in mouse brain tissue lysate.

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

Exposure time: 3.25 secs.
All lanes:
Immunoprecipitation - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - Neuronal Marker (ab254348)

Predicted band size: 102 kDa

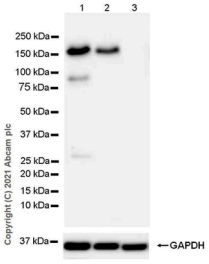
Observed band size: 160 kDa



ELISA - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
ELISA analysis using ab254348 at a range of 0-1000 ng/ml followed by a Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/2500 dilution. Antigen concentration: 1000 ng/ml.

Antigens: Mouse 160 kD Neurofilament Medium.



Western blot - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

160 kD Neurofilament Medium Western blot staining using rabbit Anti-160 kD Neurofilament Medium antibody

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The molecular weight is consistent to what has been described in the literature (PMID:19239416, PMID:27000625).

Negative control: liver (PMID:30541916).

Exposure time: 10 secs.

All lanes:
Western blot - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - Neuronal Marker (ab254348) at 1/1000 dilution

Lane 1:
Human cerebellum tissue lysate at 20 µg

Lane 2:
Human nerve tissue lysate at 20 µg

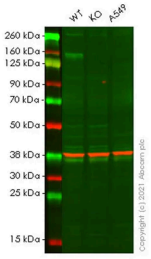
Lane 3:
Human liver tissue lysate at 20 µg

Secondary

All lanes:
Western blot - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/1000 dilution

Predicted band size: 102 kDa

Observed band size: 160 kDa



Western blot - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

160 kD Neurofilament Medium Western blot staining using rabbit Anti-160 kD Neurofilament Medium antibody

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: Intercept® (TBS) Blocking Buffer diluted with an equal volume of 0.1% TBS.

Lanes 1-3: Merged signal (red and green). Green - ab254348 observed at 160 kDa. Red - loading control ab8245 observed at 36 kDa.

ab254348 Anti-NEFM antibody [EPR23510-76] was shown to specifically react with NEFM in wild-type HEK-293T cells. Loss of signal was observed when the knockout cell line ab266741 (knockout cell lysate ab257103) was used. Wild-type and NEFM knockout samples were subjected to SDS-PAGE. ab254348 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at at 4°C overnight at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - Neuronal Marker (ab254348) at 1/1000 dilution

Lane 1:
Wild-type HEK-293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 2:
NEFM knockout HEK-293T whole cell lysate at 20 µg

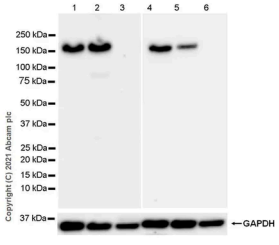
Lane 3:
A549 (human lung carcinoma epithelial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) at 1/10000 dilution

Predicted band size: 102 kDa

Observed band size: 160 kDa



Western blot - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - BSA and Azide free (ab281830)

160 kD Neurofilament Medium Western blot staining using rabbit Anti-160 kD Neurofilament Medium antibody

This data was developed using ab254348, the same antibody clone in a different buffer formulation.
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

The molecular weight is consistent to what has been described in the literature (PMID:19239416, PMID:27000625.

Negative control: liver (PMID:30541916).

Exposure times: Lane 1-3: 3.25 secs; Lane 4-6: 10 secs.
All lanes:
Western blot - Anti-160 kD Neurofilament Medium antibody [EPR23510-76] - Neuronal Marker (ab254348) at 1/1000 dilution

Lane 1:
Mouse brain tissue lysate at 20 µg

Lane 2:
Mouse cerebellum tissue lysate at 20 µg

Lane 3:
Mouse liver tissue lysate at 20 µg

Lane 4:
Rat brain tissue lysate at 20 µg

Lane 5:
Rat cerebellum tissue lysate at 20 µg

Lane 6:
Rat liver tissue lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 102 kDa

Observed band size: 160 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.