# abcam

### Product datasheet

## Anti-68kDa Neurofilament/NF-L antibody [DA2] ab7255

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Overview

Product name Anti-68kDa Neurofilament/NF-L antibody [DA2]

**Description**Mouse monoclonal [DA2] to 68kDa Neurofilament/NF-L

Host species Mouse

Specificity Specifically recognizes the light neurofilament subunit or 68kDa Neurofilament/NF-L.

Tested applications Suitable for: Flow Cyt, ICC/IF, WB, IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Bird, Mammals 4

Immunogen Full length native protein (purified) corresponding to Pig 68kDa Neurofilament/NF-L. A

preparation of enzymatically dephosphorylated pig neurofilaments including NF-L, NF-M and NF-H. Screening was by ELISA on the immunogen followed by immunofluorescence microscopy.

**Epitope** The epitope for this antibody is not in the N-terminus; however exactly where it is located is not

known. It is thought to be somewhere within the central alpha helical "rod" region of the molecule.

General notes

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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -

80°C. Avoid freeze / thaw cycle.

Storage buffer Preservative: 0.033% Sodium azide

Constituent: Tissue culture supernatant

**Purity** Tissue culture supernatant

**Clonality** Monoclonal

Clone number DA2

1

**Light chain type** lgG1 kappa

#### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab7255 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		1/100.  ab170190 - Mouse monoclonal lgG1, is suitable for use as an isotype control with this antibody.
ICC/IF	★★☆☆☆(1)	1/1000. For unpurifed use at : 1/100 - 1/500
WB		1/5000 - 1/10000.
IHC-P		1/1000. (works on mildly formalin fixed sections)

#### **Target**

#### **Function**

Neurofilaments usually contain three intermediate filament proteins: L, M, and H which are involved in the maintenance of neuronal caliber.

#### Involvement in disease

Defects in NEFL are the cause of Charcot-Marie-Tooth disease type 1F (CMT1F) [MIM:607734]. CMT1F is a form of Charcot-Marie-Tooth disease, the most common inherited disorder of the peripheral nervous system. Charcot-Marie-Tooth disease is classified in two main groups on the basis of electrophysiologic properties and histopathology: primary peripheral demyelinating neuropathy or CMT1, and primary peripheral axonal neuropathy or CMT2. Neuropathies of the CMT1 group are characterized by severely reduced nerve conduction velocities (less than 38 m/sec), segmental demyelination and remyelination with onion bulb formations on nerve biopsy, slowly progressive distal muscle atrophy and weakness, absent deep tendon reflexes, and hollow feet. CMT1F is characterized by onset in infancy or childhood (range 1 to 13 years).

Defects in NEFL are the cause of Charcot-Marie-Tooth disease type 2E (CMT2E) [MIM:607684]. CMT2E is an autosomal dominant form of Charcot-Marie-Tooth disease type 2. Neuropathies of the CMT2 group are characterized by signs of axonal regeneration in the absence of obvious myelin alterations, normal or slightly reduced nerve conduction velocities, and progressive distal muscle weakness and atrophy.

#### Sequence similarities

Belongs to the intermediate filament family.

**Domain** 

The extra mass and high charge density that distinguish the neurofilament proteins from all other intermediate filament proteins are due to the tailpiece extensions. This region may form a charged scaffolding structure suitable for interaction with other neuronal components or ions.

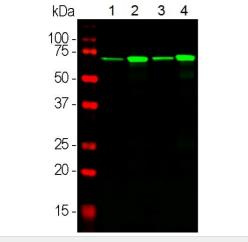
# Post-translational modifications

O-glycosylated.

Phosphorylated in the Head and Rod regions by the PKC kinase PKN1, leading to inhibit

polymerization.

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(ab7255) at 1/5000 dilution

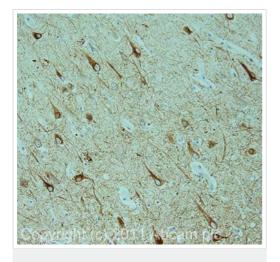
Lane 1: rat brain whole tissue lysates

Lane 2: rat spinal cord whole tissue lysates Lane 3: mouse brain whole tissue lysates

Lane 4: mouse spinal cord whole tissue lysates

All lanes: Anti-68kDa Neurofilament/NF-L antibody [DA2]

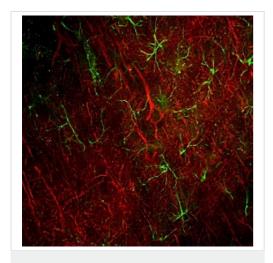
Western blot - Anti-68kDa Neurofilament/NF-L antibody [DA2] (ab7255)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-68kDa Neurofilament/NF-L antibody [DA2] (ab7255)

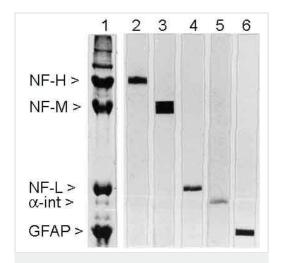
IHC image of ab7255 staining in human hippocampus formalin fixed paraffin embedded tissue section, performed on a Leica Bond<sup>TM</sup> system using the standard protocol F. The section was pretreated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab7255, 1µg/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-68kDa Neurofilament/NF-L antibody [DA2] (ab7255)

Ab7255 staining 68kDa Neurofilament/NF-L in rat frontal cortex sections by Immunocytochemistry/Immunofluorescence. Samples were incubated with primary antibody at 1/5000 dilution (red) and chicken polyclonal to GFAP.



Western blot - Anti-68kDa Neurofilament/NF-L antibody [DA2] (ab7255)

Lane 1 : Coomassie Blue stain

Lane 2: Heavy neurofilament antibody

Lane 3: Anti-160 kD Neurofilament Medium antibody [3H11]

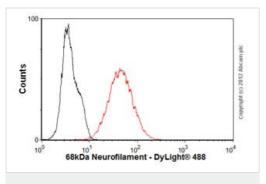
(ab7256)

Lane 4: Anti-68kDa Neurofilament/NF-L antibody [DA2] (ab7255)

Lane 5: alpha Internexin antibody

Lane 6: GFAP antibody

All lanes: Cytoskeletal homogenates of rat spinal cord



Flow Cytometry - Anti-68kDa Neurofilament/NF-L antibody [DA2] (ab7255)

Overlay histogram showing SH-SY5Y cells stained with ab7255 (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 (ab7255, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10<sup>6</sup> cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in SH-SY5Y cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

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