# abcam

# Product datasheet

# Anti-160 kD Neurofilament Medium antibody [NF-09] - Neuronal Marker ab7794



★★★★★ 5 Abreviews 47 References 画像数 6

#### 製品の概要

製品名 Anti-160 kD Neurofilament Medium antibody [NF-09] - Neuronal Marker

製品の詳細 Mouse monoclonal [NF-09] to 160 kD Neurofilament Medium - Neuronal Marker

由来種 Mouse

特異性 This antibody reacts with both phosphorylated and non-phosphorylated forms of medium

neurofilament protein (160 kDa) of various species.

アプリケーション 適用あり: WB, ELISA, ICC/IF, IHC-Fr, IHC-P, IHC (PFA fixed)

**種交差性 交差種:** Mouse, Rat, Cow, Cat, Human, Pig

交差が予測される動物種: a wide range of other species, Mammals 4

免疫原 Pellet of pig brain cold stable proteins after depolymerization of microtubules.

ポジティブ・コントロール WB: HEK-293 and A549 whole cell lysates ICC/IF: Dental pulp stem, Neuro2A, PC12 and human

nerve cells

特記事項

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

製品の特性

製品の状態 Liquid

保存方法 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.

**バッファー** pH: 7.40

Preservative: 0.097% Sodium azide

Constituent: PBS

精製度 Protein A purified

1

ポリ/モノ モノクローナル

 クローン名
 NF-09

 アイソタイプ
 IgG2a

 軽鎖の種類
 unknown

## アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab7794の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use a concentration of 1 - 2 μg/ml.
ELISA		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IHC-Fr	<b>★★★★</b> (1)	Use at an assay dependent concentration.
IHC-P	**** (1)	Use at an assay dependent concentration.
IHC (PFA fixed)		Use at an assay dependent concentration.

# ターゲット情報

機能 Neurofilaments usually contain three intermediate filament proteins: L, M, and H which are

involved in the maintenance of neuronal caliber.

**配列類似性** Belongs to the intermediate filament family.

翻訳後修飾 There are a number of repeats of the tripeptide K-S-P, NFM is phosphorylated on a number of the

serines in this motif. It is thought that phosphorylation of NFM results in the formation of interfilament cross bridges that are important in the maintenance of axonal caliber.

Phosphorylation seems to play a major role in the functioning of the larger neurofilament

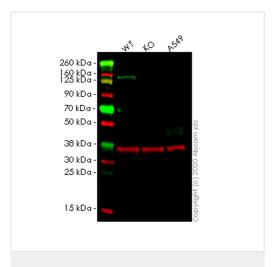
polypeptides (NF-M and NF-H), the levels of phosphorylation being altered developmentally and

coincidentally with a change in the neurofilament function.

Phosphorylated in the head and rod regions by the PKC kinase PKN1, leading to the inhibition of

polymerization.

# 画像



Western blot - Anti-160 kD Neurofilament Medium antibody [NF-09] - Neuronal Marker (ab7794)

**All lanes :** Anti-160 kD Neurofilament Medium antibody [NF-09] - Neuronal Marker (ab7794) at 1/1000 dilution

Lane 1: Wild-type HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lane 2: NEFM knockout HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lane 3: A549 (Human lung carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

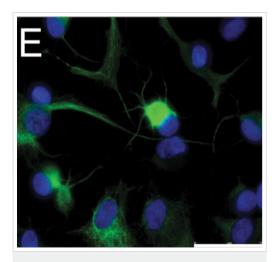
#### Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (IRDye® 680RD) preadsorbed (<u>ab216777</u>) at 1/10000 dilution

Observed band size: 150 kDa

**Lanes 1-3:** Merged signal (red and green). Green - ab7794 observed at 150 kDa. Red - loading control **ab181602** observed at 36 kDa.

ab7794 Anti-160 kD Neurofilament Medium antibody [NF-09] was shown to specifically react with 160 kD Neurofilament Medium in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line <a href="mailto:ab266741">ab266741</a> (knockout cell lysate <a href="mailto:ab257103">ab257103</a>) was used. Wild-type and 160 kD Neurofilament Medium knockout samples were subjected to SDS-PAGE. ab7794 and Anti-GAPDH antibody[EPR16891] - Loading Control (<a href="mailto:ab181602">ab181602</a>) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 680RD) preadsorbed (<a href="mailto:ab216777">ab216777</a>) and Goat anti-Mouse lgG H&L (IRDye® 800CW) preadsorbed (<a href="mailto:ab216772">ab216772</a>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

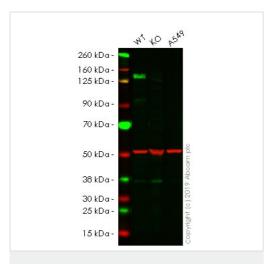


Immunocytochemistry/ Immunofluorescence - Anti-160 kD Neurofilament Medium antibody [NF-09] -Neuronal Marker (ab7794)

Image from Ferro F et al., PLoS One. 2012;7(7):e41774. Epub 2012 Jul 23. Fig 3.; doi:10.1371/journal.pone.0041774; July 23, 2012, PLoS ONE 7(7): e41774.

Immunofluorescence analysis of 1 month neuronal-differentiated dental pulp stem cells, staining 160 kD Neurofilament Medium with ab7794.

Cells were fixed with paraformaldehyde and incubated with primary antibody (1/600). A FITC-conjugated anti-mouse lgG (1/375) was used as the secondary antibody.



Western blot - Anti-160 kD Neurofilament Medium antibody [NF-09] - Neuronal Marker (ab7794)

**All lanes :** Anti-160 kD Neurofilament Medium antibody [NF-09] - Neuronal Marker (ab7794) at 1/1000 dilution

Lane 1: Wild-type HEK-293 whole cell lysate

Lane 2: NEFM knockout HEK-293 whole cell lysate

Lane 3: A549 whole cell lysate

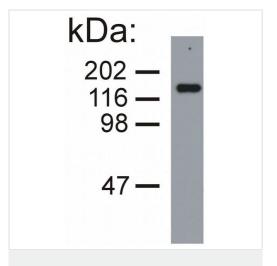
Lysates/proteins at 20 µg per lane.

Observed band size: 150 kDa

**Lanes 1 - 4:** Merged signal (red and green). Green - ab7794 observed at 150 kDa. Red - loading control, **ab52866**, observed at 50 kDa.

ab7794 was shown to specifically react with NEFM (Neurofilament) in wild-type HEK-293 cells as signal was lost in NEFM knockout cells. Wild-type and NEFM knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% NF Milk. Ab7794 and <a href="mailto:ab52866">ab52866</a> (Rabbit anti alpha Tubulin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed <a href="mailto:ab216772">ab216772</a> and Goat anti-

Rabbit IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed **ab216777** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

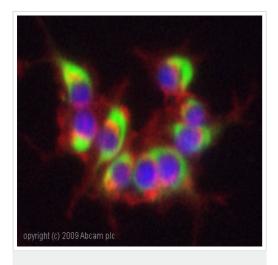


Western blotting analysis of neurofilament medium protein in porcine brain lysate (reducing conditions) by mouse monoclonal **NF-09**.

Western blot - Anti-160 kD Neurofilament Medium antibody [NF-09] - Neuronal Marker (ab7794)



Immunocytochemistry/ Immunofluorescence - Anti-160 kD Neurofilament Medium antibody [NF-09] -Neuronal Marker (ab7794) ab7794 staining Neurofilament medium protein in mouse Neuro2A cells by ICC/IF.



Immunocytochemistry/ Immunofluorescence - Anti-160 kD Neurofilament Medium antibody [NF-09] -Neuronal Marker (ab7794)

ICC/IF image of ab7794 stained PC12 cells. The cells were 100% methanol fixed (5 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 (ab7794, 1æg/ml) overnight at +4øC. The secondary antibody (green)ÿwas Alexa Fluor© 488 goat anti-mouse IgG (H+L) used at a 1/1000 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 1.43æM.

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