# abcam

## Product datasheet

## Anti-CNPase antibody [11-5B] ab6319



★★★★★ 38 Abreviews 135 References 画像数 10

#### 製品の概要

製品名 Anti-CNPase antibody [11-5B]

製品の詳細 Mouse monoclonal [11-5B] to CNPase

由来種 Mouse

アプリケーション 適用あり: IHC-P, WB, ICC/IF 種交差性 交差種: Mouse, Rat, Human

交差が予測される動物種: Sheep, Rabbit, Cow, Dog, Pig, Rhesus monkey 4 非交差種:

Chicken, Guinea pig

免疫原 Full length native protein (purified) corresponding to Human CNPase.

ポジティブ・コントロール WB: HeLa and Hap1 cell lysates; Human and Mouse Spinal Cord and Brain tissue lysates; Rat

Brain tissue lysate. IHC-P: FFPE human cerebral cortex tissue sections. ICC: Primary

hippocampal rat neurons/glia, DIV14. cells

**特記事項**This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or

conjugation for your experiments, please contact orders@abcam.com.

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: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine

1

精製度 Protein G purified

**ポリ/モノ** モノクローナル

**クローン名** 11-5B

アイソタイプ IgG1

#### 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ab6319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
IHC-P	<b>★★★★★ (15)</b>	Use at an assay dependent concentration.
WB	<b>★★★★★ (8)</b>	Use a concentration of 5 µg/ml. Detects a band of approximately 48 kDa (predicted molecular weight: 48 kDa).
ICC/IF	<b>★★★★★</b> (7)	Use at an assay dependent concentration.

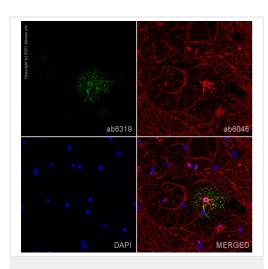
#### ターゲット情報

**配列類似性** Belongs to the cyclic nucleotide phosphodiesterase family.

**細胞内局在** Membrane. Melanosome. Firmly bound to membrane structures of brain white matter. Identified

by mass spectrometry in melanosome fractions from stage I to stage  $\ensuremath{\mathsf{I}}$  V.

#### 画像

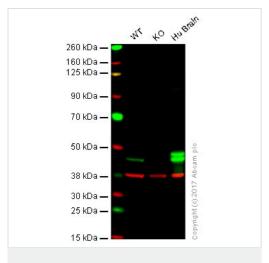


Immunocytochemistry/ Immunofluorescence - Anti-CNPase antibody [11-5B] (ab6319)

ab6319 staining CNPase in primary hippocampal rat neurons/glia, (obtained from Neuromics, cat. no. PC35101), DIV14. cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab6319 at 1µg/ml and ab6046, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with ab150117, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and ab150080, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.



Western blot - Anti-CNPase antibody [11-5B] (ab6319)

All lanes: Anti-CNPase antibody [11-5B] (ab6319) at 5 µg/ml

Lane 1: Wild-type HAP1 whole cell lysate at 40 µg

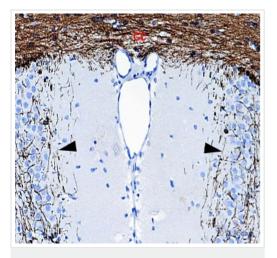
Lane 2 : CNPase knockout HAP1 whole cell lysate at 40  $\mu g$ 

Lane 3: Human Brain whole cell lysate at 20 µg

Predicted band size: 48 kDa

**Lanes 1 - 3:** Merged signal (red and green). Green - ab6319 observed at 48 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

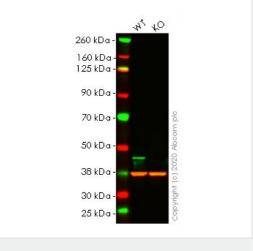
ab6319 was shown to recognize CNPase in wild-type HAP1 cells as signal was lost at the expected MW in CNPase knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and CNPase knockout samples were subjected to SDS-PAGE. Ab6319 and <a href="mailto:ab181602">ab181602</a> (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at 5 µg/ml 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® 680RD) preabsorbed <a href="mailto:ab216777">ab216777</a> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CNPase antibody [11-5B] (ab6319)

This image is courtesy of an abreview submitted by Carl Hobbs, King's College London, United Kingdom

IHC-P image of CNPase staining on rat brain sections using ab6319 (1/1600). heat mediated antigen retrieval on paraffin embedded sections was performed using citric acid. The sections were then blocked with 1% BSA for 10 min at 21°C. The primary antibody was incubated for 16 hours at 21°C. The sections were then incubated in Goat anti-mouse (Biotin) at 1:200.



Western blot - Anti-CNPase antibody [11-5B] (ab6319)

All lanes: Anti-CNPase antibody [11-5B] (ab6319) at 5 μg/ml

Lane 1: Wild-type HeLa cell lysate

Lane 2: CNP knockout HeLa cell lysate

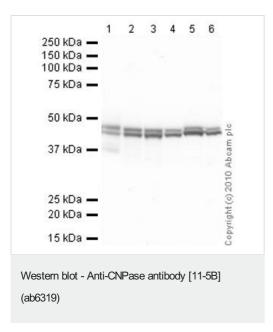
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 48 kDa **Observed band size:** 48 kDa

**Lanes 1-2:** Merged signal (red and green). Green - ab6319 observed at 48 kDa. Red - Anti-GAPDH antibody[EPR16891] - Loading Control (ab181602) observed at 37 kDa.

ab6319 was shown to react with CNPase in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab264949 (knockout cell lysate ab256877) was used. Wild-type HeLa and CNP knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab6319 and Anti-GAPDH antibody[EPR16891] - Loading Control (ab181602) overnight at 4°C at a 5 µg/ml and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse lgG H&L (IRDye®800CW) preadsorbed (ab216772) and Goat Anti-Rabbit lgG H&L (IRDye®680RD) preadsorbed (ab216777) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



**All lanes :** Anti-CNPase antibody [11-5B] (ab6319) at 1/100 dilution

Lane 1: Human spinal cord tissue lysate - total protein (ab29188)

Lane 2 : Human brain tissue lysate - total protein (ab29466)

Lane 3: Spinal Cord (Mouse) Tissue Lysate

Lane 4: Brain (Mouse) Tissue Lysate

Lane 5: Spinal Cord (Rat) Tissue Lysate

Lane 6: Brain (Rat) Tissue Lysate

Lysates/proteins at 20 µg per lane.

#### **Secondary**

**All lanes :** Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

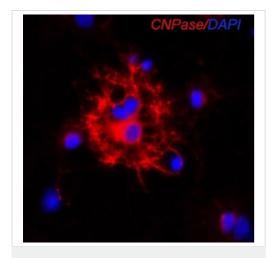
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 48 kDa

Exposure time: 1 minute

This antibody was raised against full length native CNPase and is predicted to recognize both isoforms. The predicted molecular weights of isoforms CNPI and CNPII are 45- and 48-kDa respectively.

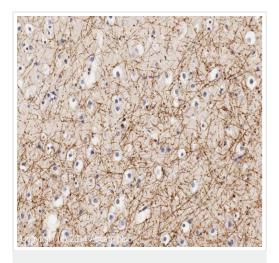


Immunocytochemistry/ Immunofluorescence - Anti-CNPase antibody [11-5B] (ab6319)

This image is courtesy of an anonymous abreview.

ab6319 staining CNPase in rat oligodendrocytes by Immunocytochemistry/ Immunofluorescence.

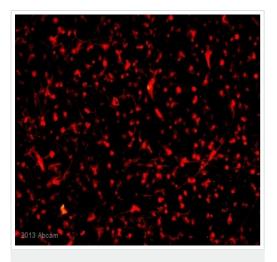
Cells were fixed in paraformaldehyde, blocked using 5% serum for 10 minutes at 25°C, then incubated with ab6319 at a 1/200 dilution for 2 hours at 25°C. The secondary used was a goat anti-mouse Cy3 conjugated polyclonal at a 1/100 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CNPase antibody [11-5B] (ab6319)

IHC image of CNPase staining in human cerebral cortex formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ 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 ab6319, 5µg/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.

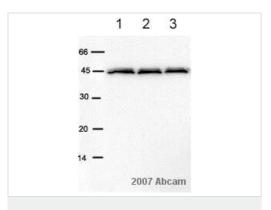
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunocytochemistry/ Immunofluorescence - Anti-CNPase antibody [11-5B] (ab6319)

This image is courtesy of an anonymous Abreview

ab6319 staining CNPase in the rat oligodendrocytes by ICC/IF (Immunoytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with methanol and blocked with 5% BSA for 1 hour at 37°C. Samples were incubated with primary antibody (1/100 in PBS) for 18 hours at 4°C. An Alexa Fluor<sup>®</sup> 594-conjugated Goat anti-mouse IgG polyclonal (1:200) was used as the secondary antibody.



Western blot - Anti-CNPase antibody [11-5B] (ab6319)

This image is courtesy of an anonymous Abreview

**All lanes :** Anti-CNPase antibody [11-5B] (ab6319) at 1/750 dilution

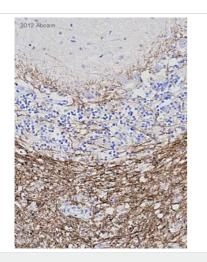
All lanes: Spinal Cord homogenate (whole tissue lysate)

Lysates/proteins at 2 µg per lane.

## Secondary

All lanes: HRP conjugated sheep anti-mouse IgG

**Predicted band size:** 48 kDa **Observed band size:** 45,47 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CNPase antibody [11-5B] (ab6319)

This image is courtesy of an abreview submitted by Carl Hobbs, King's College London, United Kingdom

ab6319 staining CNPase in Dog Cerebellum tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections). Tissue was fixed with formaldehyde and blocked with 1% BSA for 10 minutes at 21°C; antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with primary antibody (1/1500 in blocking buffer) for 2 hours at 21°C. A Biotin-conjugated Goat anti-mouset IgG polyclonal (1/200) was used as the secondary antibody.

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