abcam

Product datasheet

Anti-nNOS (neuronal) antibody [EP1855Y] ab76067

Recombinant RabMAb

★★★★★ 10 Abreviews 63 References 10 Images

Overview

Product name Anti-nNOS (neuronal) antibody [EP1855Y]

Description Rabbit monoclonal [EP1855Y] to nNOS (neuronal)

Host species Rabbit

Tested applications Suitable for: Flow Cyt (Intra), WB, IP, ICC/IF

Unsuitable for: IHC-P

Species reactivity Reacts with: Mouse. Rat

Predicted to work with: Human, Common marmoset

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Mouse brain tissue lysate. ICC/IF: PC-12 cells. Flow Cyt (intra): PC-12 cells. IP: Rat brain

tissue lysate.

General notes 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 production

For more information see here.

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

Properties

Form

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

Avoid freeze / thaw cycle.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EP1855Y

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab76067 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 (Intra)		1/600. For unpurified use at 1/15. <u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (6)	1/1000 - 1/2000. Predicted molecular weight: 161 kDa. For unpurified use at 1/100.
IP		1/150. For unpurified use at 1/4.
ICC/IF	★★★★★ (1)	1/200.

Application notes

Is unsuitable for IHC-P.

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Function Produces nitric oxide (NO) which is a messenger molecule with diverse functions throughout the

body. In the brain and peripheral nervous system, NO displays many properties of a

neurotransmitter. Probably has nitrosylase activity and mediates cysteine S-nitrosylation of

cytoplasmic target proteins such SRR.

Tissue specificity Isoform 1 is ubiquitously expressed: detected in skeletal muscle and brain, also in testis, lung and

kidney, and at low levels in heart, adrenal gland and retina. Not detected in the platelets. Isoform 3 is expressed only in testis. Isoform 4 is detected in testis, skeletal muscle, lung, and kidney, at low

levels in the brain, but not in the heart and adrenal gland.

Sequence similarities Belongs to the NOS family.

Contains 1 FAD-binding FR-type domain.

Contains 1 flavodoxin-like domain.

Contains 1 PDZ (DHR) domain.

Domain The PDZ domain in the N-terminal part of the neuronal isoform participates in protein-protein

interaction, and is responsible for targeting nNos to synaptic membranes in muscles. Mediates

interaction with VAC14.

Post-translational

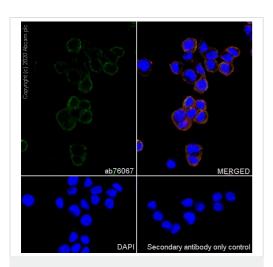
modifications

Ubiquitinated; mediated by STUB1/CHIP in the presence of Hsp70 and Hsp40 (in vitro).

Cellular localization Cell membrane > sarcolemma. Cell projection > dendritic spine. In skeletal muscle, it is localized

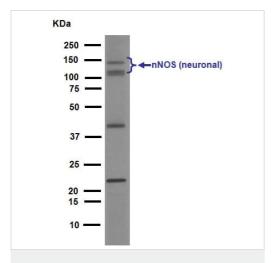
beneath the sarcolemma of fast-twitch muscle fiber by associating with the dystrophin

glycoprotein complex. In neurons, enriched in dendritic spines.



Immunocytochemistry/ Immunofluorescence - AntinNOS (neuronal) antibody [EP1855Y] (ab76067)

Immunocytochemistry analysis of PC-12 cells labelling nNOS (neuronal) (green) with purified ab76067 at 1/250. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. Cells were counterstained with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) at 1/200 dilution (red). Nuclear DNA was labelled with DAPI (blue).



Western blot - Anti-nNOS (neuronal) antibody [EP1855Y] (ab76067)

Anti-nNOS (neuronal) antibody [EP1855Y] (ab76067) at 1/100 dilution (unpurified) + Mouse brain tissue lysate at 10 µg

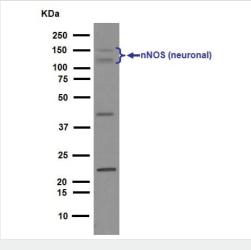
Secondary

Peroxidase-conjugated goat anti-rabbit lgG (H+L) at 1/1000 dilution

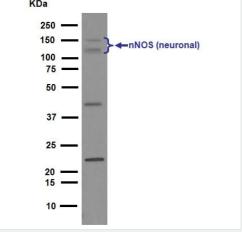
Predicted band size: 161 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

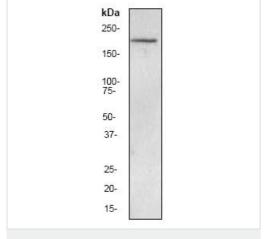
Diluting buffer and concentration: 5% NFDM /TBST.



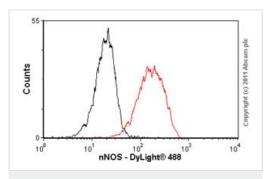
Western blot - Anti-nNOS (neuronal) antibody



[EP1855Y] (ab76067)



Western blot - Anti-nNOS (neuronal) antibody [EP1855Y] (ab76067)



Flow Cytometry (Intracellular) - Anti-nNOS (neuronal) antibody [EP1855Y] (ab76067)

Anti-nNOS (neuronal) antibody [EP1855Y] (ab76067) at 1/3000 dilution (purified) + Mouse brain tissue lysate at 10 µg

Secondary

Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 161 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

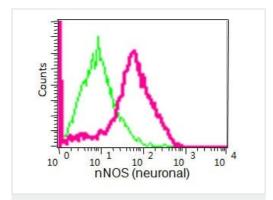
Anti-nNOS (neuronal) antibody [EP1855Y] (ab76067) at 1/1000 dilution (unpurified) + Mouse brain tissue lysate at 10 µg

Secondary

HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

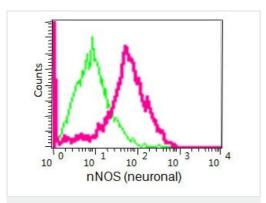
Predicted band size: 161 kDa Observed band size: 161 kDa

Overlay histogram showing PC-12 cells stained with unpurified ab76067 (red line). The cells were fixed with methanol (5 min) and incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (unpurified ab76067, 1/50 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit monoclonal IgG (1µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a slightly decreased signal in PC-12 cells fixed with 4% paraformaldehyde (10 min) used under the same conditions. Please note that Abcam do not have any data for use of this antibody in non-fixed cells. We welcome any customer feedback.



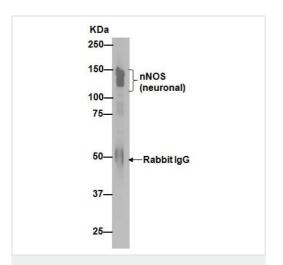
Flow Cytometry (Intracellular) - Anti-nNOS (neuronal) antibody [EP1855Y] (ab76067)

Intracellular Flow Cytometry analysis of PC-12 cells labelling nNos (neuronal) with unpurified ab76067 at 1/15 (red). Cells were fixed with 100% methanol. A FITC-conjugated goat anti-rabbit lgG (1/150) was used as the secondary antibody. Green - Isotype control, rabbit monoclonal lgG.



Flow Cytometry (Intracellular) - Anti-nNOS (neuronal) antibody [EP1855Y] (ab76067)

Intracellular Flow Cytometry analysis of PC-12 cells labelling nNos (neuronal) with purified ab76067 at 1/600 (red). Cells were fixed with 100% methanol. A FITC-conjugated goat anti-rabbit lgG (1/150) was used as the secondary antibody. Green - Isotype control, rabbit monoclonal lgG.

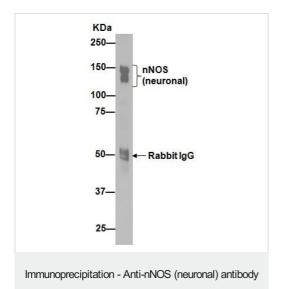


Immunoprecipitation - Anti-nNOS (neuronal) antibody [EP1855Y] (ab76067)

ab76067 (unpurified) at 1/4 immunoprecipitating nNOS (neuronal) in rat brain tissue lysate. For western blotting, a peroxidase-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/1000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

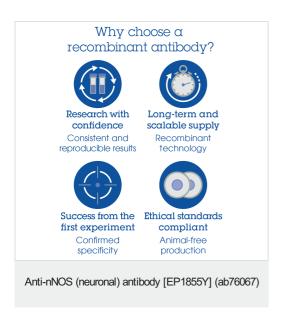


[EP1855Y] (ab76067)

ab76067 (purified) at 1/150 immunoprecipitating nNOS (neuronal) in rat brain tissue lysate. For western blotting, a peroxidase-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/1000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



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