abcam

Product datasheet

Anti-nNOS (neuronal) (phospho S847) antibody ab16650

★★★★★ 4 Abreviews   11 References   5 Images

Overview

Product name	Anti-nNOS (neuronal) (phospho S847) antibody
Description	Rabbit polyclonal to nNOS (neuronal) (phospho S847)
Host species	Rabbit
Specificity	This antibody shows a reduction in signal when blocked with unmodified nNOS (neuronal) peptide in WB, however when tested in ELISA, it showed less than 2% cross reactivity with the unmodified protein.
Tested applications	**Suitable for:** IHC-Fr, IHC-FoFr, WB
Species reactivity	**Reacts with:** Mouse, Rat, Apteronotus leptorhynchus
	Predicted to work with: Rabbit, Human, Xenopus laevis, Zebrafish ⚠
Immunogen	Synthetic peptide conjugated to KLH derived from within residues 800 - 900 of Mouse nNOS (neuronal), phosphorylated at S847. Read Abcam's proprietary immunogen policy (Peptide available as ab16981.)
Positive control	This antibody gave a positive signal in mouse forebrain and mouse spinal cord tissue lysates. HeLa whole cell lysate was used as a negative control. nNOS is widely expressed in the nervous system: cerebrum, olfactory bulb, hippocampus, midbrain, cerebellum, pons, medulla oblongata, and spinal cord. It is also found in skeletal muscle, spleen, heart, kidney, and liver.

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: 0.02% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab16650** 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
IHC-Fr	★★★★★	Use at an assay dependent concentration.
IHC-FoFr	★★★★★	1/3000.
WB	★★★★☆	Use a concentration of 1 μg/ml. Detects a band of approximately 160 kDa (predicted molecular weight: 160 kDa).

Target

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.

Images
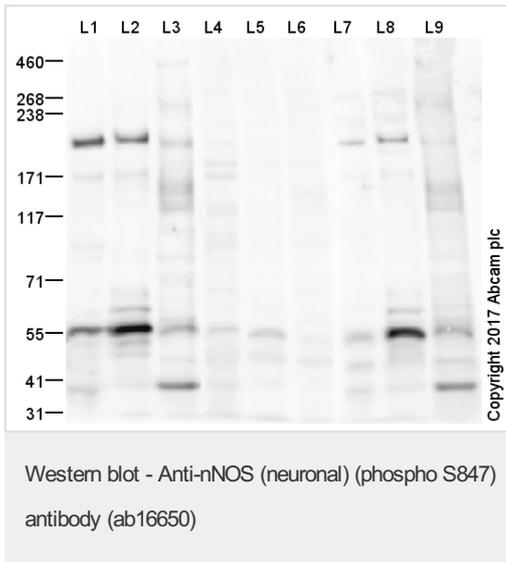
Western blot - Anti-nNOS (neuronal) (phospho S847) antibody (ab16650)

All lanes : Anti-nNOS (neuronal) (phospho S847) antibody (ab16650) at 1 µg/ml

Lane 1 : Forebrain (Mouse) Tissue Lysate

Lane 2 : Spinal Cord (Mouse) Tissue Lysate

Lane 3 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate (negative control)

Lane 4 : Forebrain (Mouse) Tissue Lysate with Mouse nNOS (neuronal) (phospho S847) peptide at 1 µg/ml

Lane 5 : Spinal Cord (Mouse) Tissue Lysate with Mouse nNOS (neuronal) (phospho S847) peptide at 1 µg/ml

Lane 6 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate (negative control) with Mouse nNOS (neuronal) (phospho S847) peptide at 1 µg/ml

Lane 7 : Forebrain (Mouse) Tissue Lysate with Mouse nNOS (neuronal) (unmodified) peptide at 1 µg/ml

Lane 8 : Spinal Cord (Mouse) Tissue Lysate with Mouse nNOS (neuronal) (unmodified) peptide at 1 µg/ml

Lane 9 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate (negative control) with Mouse nNOS (neuronal) (unmodified) peptide at 1 µg/ml

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/50000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

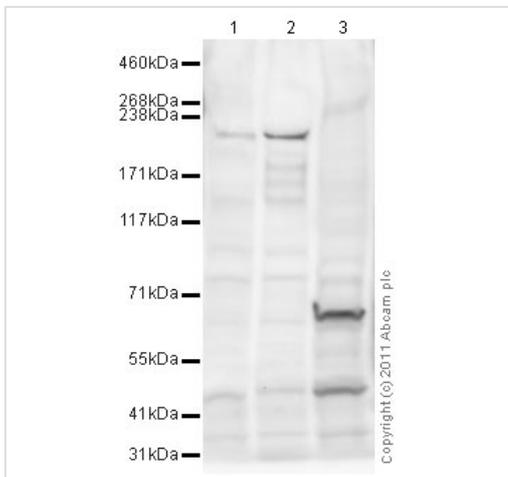**Predicted band size:** 160 kDa

Observed band size: 190 kDa

why is the actual band size different from the predicted?

Additional bands at: 55 kDa. We are unsure as to the identity of

these extra bands.

Exposure time: 4 minutes

This blot was produced using a 3-8% Tris Acetate gel under the TA buffer system. The gel was run at 150V for 60 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab16650 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution ab133406.

Western blot - Anti-nNOS (neuronal) (phospho S847) antibody (ab16650)

All lanes : Anti-nNOS (neuronal) (phospho S847) antibody (ab16650) at 1 µg/ml

Lane 1 : Forebrain (Mouse) Tissue Lysate
Lane 2 : Spinal Cord (Mouse) Tissue Lysate
Lane 3 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate (negative control)

Lysates/proteins at 20 µg per lane.

Secondary
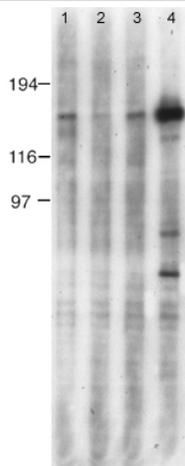**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 160 kDa
Observed band size: 190 kDa why is the actual band size different from the predicted?

Exposure time: 2 minutes

Western blot - Anti-nNOS (neuronal) (phospho S847) antibody (ab16650)

This image is courtesy of Chris Anderson, Wellcome Trust Sanger Institute, United Kingdom

Lanes 1-3 : Anti-nNOS (neuronal) (phospho S847) antibody (ab16650) at 1 µg/ml
Lane 4 : nNOS antibody at 1/2500 dilution

Lanes 1 & 4 : mouse forebrain
Lane 2 : mouse forebrain with Mouse nNOS (neuronal) (phospho S847) peptide (ab16981) at 1 µg/ml
Lane 3 : mouse forebrain with corresponding unmodified nNOS (neuronal) peptide at 1 µg/ml

Lysates/proteins at 20 µg per lane.

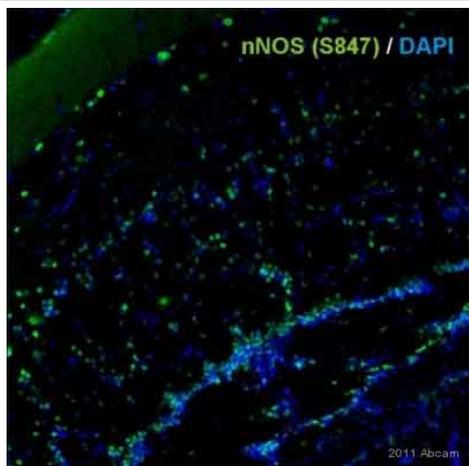**Predicted band size:** 160 kDa
Observed band size: 160 kDa

Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-nNOS (neuronal) (phospho S847) antibody (ab16650)

This image is courtesy of Sophie Pezet, CNRS, Paris, France

Immunostaining using Rabbit polyclonal to nNOS (neuronal) (phospho S847) (ab16650) on rat brain tissue sections (30 micron free floating). ab16650 was used at a dilution of 1/3000 and incubated for 18 hours at RT (in PBS triton 0.3%). Secondary Antibody Goat anti-rabbit alexa Fluor 488 was used at a dilution of 1/1000. The image shows cytoplasmic staining of CNS neurons with ab16650 in naïve rats; the staining being observed in the soma and processes of these neurons. The staining was quenched by pre-incubation with peptide against phospho S847 (ab16981), but not by the control peptide (ab57047) indicating that ab16650 is specific for nNos phosphorylated at S847. Protocol: Rats were perfused-fixed with 4% paraformaldehyde. Tissues were post-fixed overnight in the same fixative and then cryoprotected in 20% sucrose overnight. Following embedding in OCT and freezing, tissues were cut and immunostained using the 'free floating' technique.

ab16650 staining nNOS (neuronal) (phospho S847) in 16 μm thick sections of *Apteronotus leptorhynchus* by Immunohistochemistry (Frozen sections).

Tissue was fixed in 2% paraformaldehyde, permeabilized using 0.3% Triton X-100, blocked with 3% sheep serum, 1% BSA, 1% teleostean gelatine in TBS for 1 hour at 24°C and then incubated with ab16650 at a 1/100 dilution for 18 hours at 4°C. The secondary used was an Alexa-Fluor 488 conjugated goat anti-rabbit polyclonal used at a 1/200 dilution.

Immunohistochemistry (Frozen sections) - Anti-nNOS (neuronal) (phospho S847) antibody (ab16650)

Image courtesy of Dr Ruxandra Sirbulescu by Abreview.

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