abcam

Product datasheet

Anti-NADPH oxidase 4 antibody [UOTR1B492] ab109225

Recombinant RabMAb

★★★★★ 2 Abreviews 32 References 11 Images

Overview

Product name	Anti-NADPH oxidase 4 antibody [UOTR1B492]
Description	Rabbit monoclonal [UOTR1B492] to NADPH oxidase 4
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, Flow Cyt, WB, IP, IHC-P
Species reactivity	**Reacts with:** Rat, Dog, Human
Immunogen	Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) corresponding to Human NADPH oxidase 4 aa 500 to the C-terminus. (Peptide available as ab179799)
Positive control	Fetal kidney, U87-MG, 293T, and JAR lysates Human kidney tissue
General notes	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.** This product is a recombinant rabbit monoclonal antibody.

Properties

Form	Liquid
Storage instructions	Frozen Stock (-20C). Shelf life 12 months.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 59% PBS, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	UOTR1B492
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab109225** 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
ICC/IF		1/200.
Flow Cyt		1/280. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★	1/2000. Detects a band of approximately 63 kDa (predicted molecular weight: 67 kDa).Can be blocked with NADPH oxidase 4 peptide (ab179799).
IP		1/30.
IHC-P	★★★★☆	1/250 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols. **For unpurified, use 1/100 - 1/250.**

Target

Function

Constitutive NADPH oxidase which generates superoxide intracellularly upon formation of a complex with CYBA/p22phox. Regulates signaling cascades probably through phosphatases inhibition. May function as an oxygen sensor regulating the KCNK3/TASK-1 potassium channel and HIF1A activity. May regulate insulin signaling cascade. May play a role in apoptosis, bone resorption and lipolysaccharide-mediated activation of NFKB. May produce superoxide in the nucleus and play a role in regulating gene expression upon cell stimulation. Isoform 3 is not functional. Isoform 4 displays an increased activity. Isoform 5 and isoform 6 display reduced activity.

Tissue specificity

Expressed by distal tubular cells in kidney cortex and in endothelial cells (at protein level). Widely expressed. Strongly expressed in kidney and to a lower extent in heart, adipocytes, hepatoma, endothelial cells, skeletal muscle, brain, several brain tumor cell lines and airway epithelial cells.

Sequence similarities

Contains 1 FAD-binding FR-type domain.
Contains 1 ferric oxidoreductase domain.

Developmental stage

Expressed in fetal kidney and fetal liver.

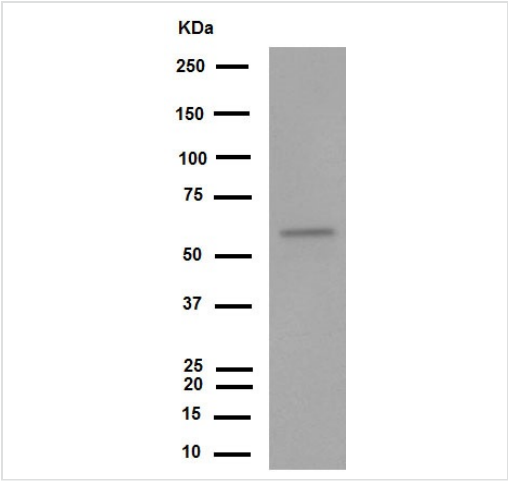
Post-translational modifications

Isoform 3 and isoform 4 are N-glycosylated. Isoform 4 glycosylation is required for its proper function.

Cellular localization

Endoplasmic reticulum membrane. Cell membrane. Cell junction > focal adhesion. Nucleus. May localize to plasma membrane and focal adhesions. According to PubMed:15927447, may also localize to the nucleus.

Images



Western blot - Anti-NADPH oxidase 4 antibody [UOTR1B492] (ab109225)

Anti-NADPH oxidase 4 antibody [UOTR1B492] (ab109225) at 1/10000 dilution (purified) + JAR cell lysate at 10 µg

Secondary

HRP goat anti-rabbit (H+L) at 1/1000 dilution

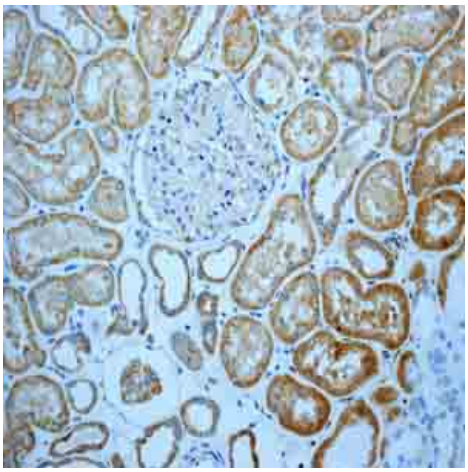
Predicted band size: 67 kDa

Observed band size: 63 kDa

why is the actual band size different from the predicted?

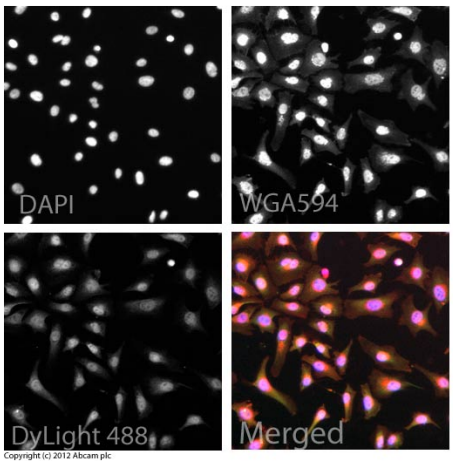
Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



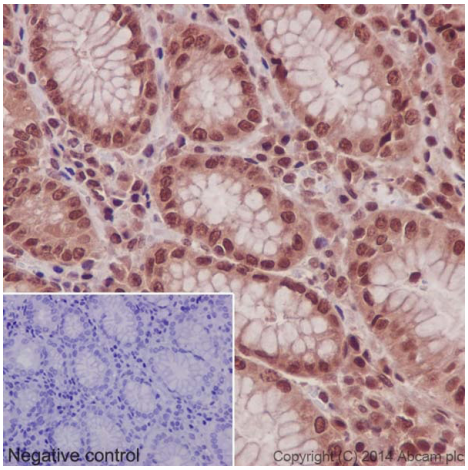
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NADPH oxidase 4 antibody [UOTR1B492] (ab109225)

Immunohistochemical analysis of paraffin-embedded Human kidney tissue using unpurified ab109225.



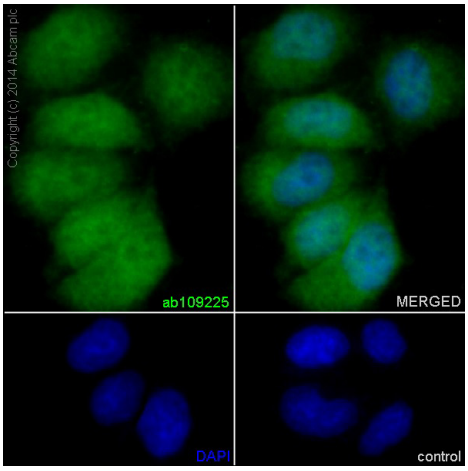
Immunocytochemistry/ Immunofluorescence - Anti-NADPH oxidase 4 antibody [UOTR1B492] (ab109225)

ICC/IF image of unpurified ab109255 stained HeLa cells. The cells were 4% formaldehyde fixed (10 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 (ab109225, 5µg/ml) overnight at +4°C. The secondary antibody (green) was ab96899, DyLight® 488 goat anti-rabbit IgG (H+L) used at a 1/250 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 1.43µM.



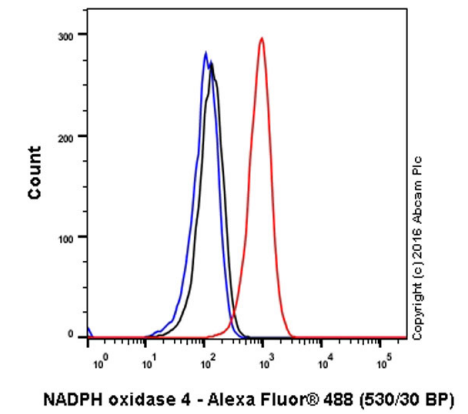
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NADPH oxidase 4 antibody [UOTR1B492] (ab109225)

Immunohistochemical staining of paraffin embedded human stomach with purified ab109225 at a dilution of 1/500. A HRP goat anti-rabbit (ab97051) was used as the secondary antibody at a dilution of 1/500 and the sample was counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



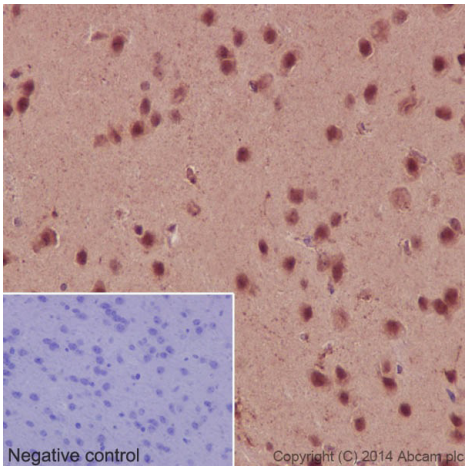
Immunocytochemistry/ Immunofluorescence - Anti-NADPH oxidase 4 antibody [UOTR1B492] (ab109225)

Immunofluorescent staining of HeLa cells (fixed in 4% PFA, permeabilized with 0.1% Triton X 100) using purified ab109225 at a dilution of 1/200. An Alexa Fluor® 488 goat anti-rabbit antibody was used as the secondary at a dilution of 1/500 and the cells were counter stained with DAPI. The negative control is shown in the bottom right hand panel - for the negative control, Alex Fluor® 594 goat anti-mouse was used at a dilution of 1/500.



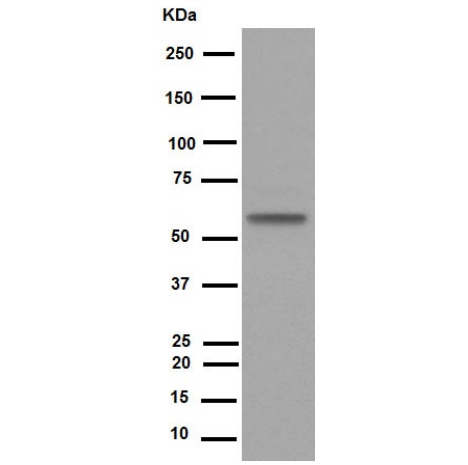
Flow Cytometry - Anti-NADPH oxidase 4 antibody [UOTR1B492] (ab109225)

Flow Cytometry analysis of U87-MG (human glioblastoma) cells labeling NADPH oxidase 4 with purified ab109225 at 1/280 dilution (10ug/mL) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-NADPH oxidase 4 antibody [UOTR1B492] (ab109225)

Immunohistochemical staining of paraffin embedded mouse cerebral cortex with purified ab109225 at a dilution of 1/500. A HRP goat anti-rabbit (ab97051) was used as the secondary antibody at a dilution of 1/500 and the sample was counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Western blot - Anti-NADPH oxidase 4 antibody [UOTR1B492] (ab109225)

Anti-NADPH oxidase 4 antibody [UOTR1B492] (ab109225) at 1/2000 dilution (purified) + Human fetal kidney at 10 µg

Secondary

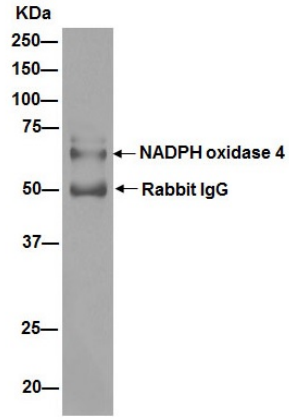
HRP anti-rabbit, specific to the non reducded form of IgG at 1/1000 dilution

Predicted band size: 67 kDa

Observed band size: 63 kDa why is the actual band size different from the predicted?

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST

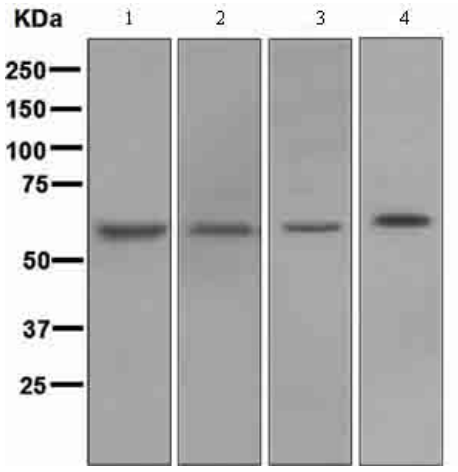


Immunoprecipitation - Anti-NADPH oxidase 4 antibody [UOTR1B492] (ab109225)

ab109225 (purified) at 1/30 immunoprecipitating NADPH oxidase 4 in HEK293. For western blotting, a HRP-conjugated anti-rabbit antibody was used as the secondary antibody (1/1000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-NADPH oxidase 4 antibody [UOTR1B492] (ab109225)

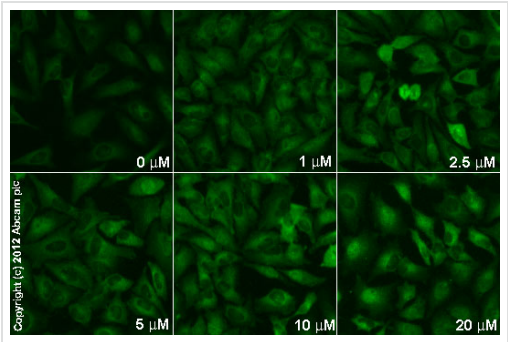
All lanes : Anti-NADPH oxidase 4 antibody [UOTR1B492] (ab109225) at 1/1000 dilution (unpurified)

Lane 1 : Fetal kidney lysate
Lane 2 : U87-MG lysate
Lane 3 : 293T lysate
Lane 4 : JAR lysates

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Standard HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 67 kDa
Observed band size: 63 kDa why is the actual band size different from the predicted?



Immunocytochemistry/ Immunofluorescence - Anti-NADPH oxidase 4 antibody [UOTR1B492] (ab109225)

Unpurified ab109225 staining Nox4 in HeLa cells treated with (-)-cannabidiol (ab120448), by ICC/IF. Increase in Nox4 expression correlates with increased concentration of (-)-cannabidiol, as described in literature.

The cells were incubated at 37°C for 6h in media containing different concentrations of ab120448 ((-)-cannabidio) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab109225 (5 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody.

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