Product Datasheet

Nox4 Antibody - BSA Free
NB110-58849

Unit Size: 0.1 ml

Store at 4C. Do not freeze.

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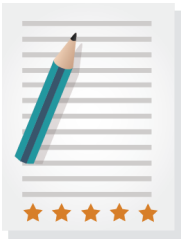
Updated 10/23/2024 v.20.1



NB110-58849

Nox4 Antibody - BSA Free

Product Information

Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description

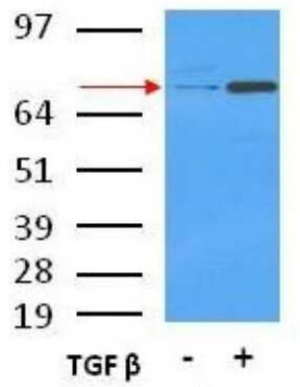
Host	Rabbit
Gene ID	50507
Gene Symbol	NOX4
Species	Human, Mouse, Rat, Porcine, Bovine, Primate, Rabbit, Sheep
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID:32853822). Porcine reactivity reported in scientific literature (PMID: 24403605).
Immunogen	A synthetic peptide made to an internal region of the human NOX4 protein (between residues 100-200) [UniProt Q9NPH5].

Product Application Details

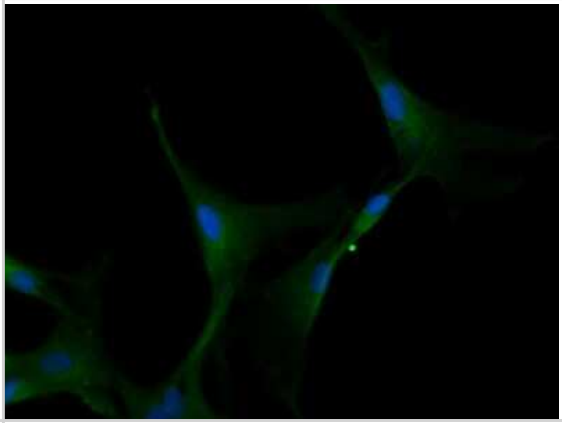
Applications	Western Blot, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Knockdown Validated
Recommended Dilutions	Western Blot 2 ug/ml, Immunohistochemistry 5 ug/ml, Immunocytochemistry/ Immunofluorescence 1:50 - 1:200, Immunohistochemistry-Paraffin 5 ug/ml, Immunohistochemistry-Frozen reported in scientific literature, Immunoblotting reported in scientific literature (PMID 28152080), Knockdown Validated
Application Notes	In Western blot this antibody recognizes a band at ~67 kDa or larger representing isoform 1 of NOX4, and what appears to be a non-specific band ~48 kDa. The observed band size may vary depending on sample type and glycosylation. In ICC/IF cytoplasmic and mitochondrial staining is observed. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended.

Images

Western Blot: Nox4 Antibody - BSA Free [NB110-58849] - Detection of NOX4 on IMR90 lysate. Image courtesy of Naomi Logsdon, University of Alabama at Birmingham.

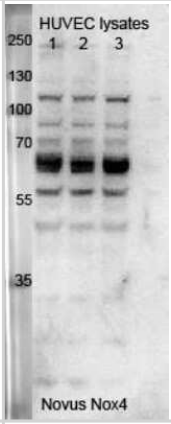


Immunocytochemistry/Immunofluorescence: Nox4 Antibody - BSA Free [NB110-58849] - Porcine aortic endothelial cells stained with NB110-58849 (green). Nuclei were counterstained with DAPI (blue). Image from verified customer review.

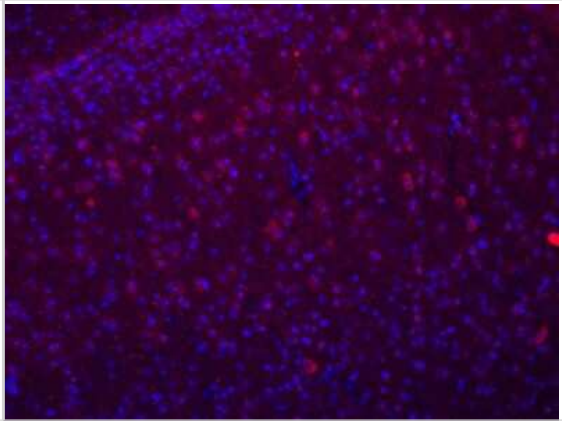


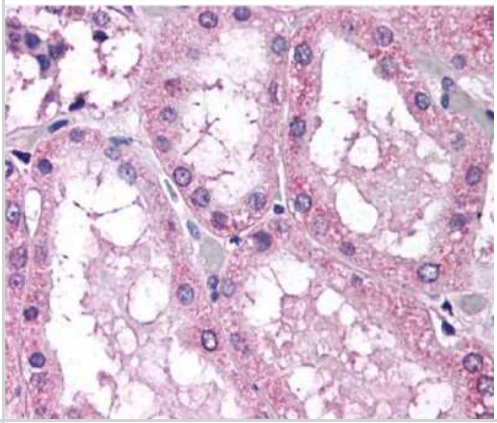
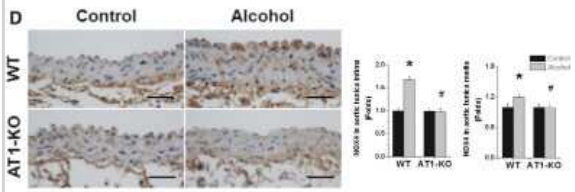
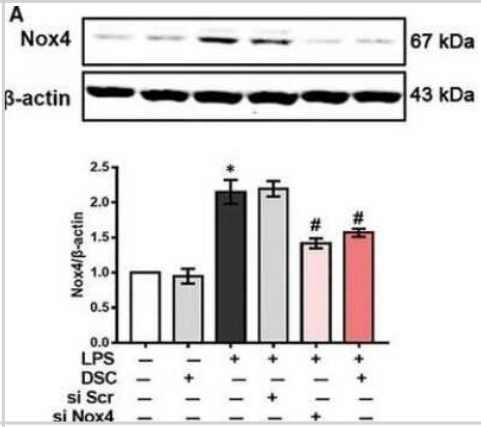
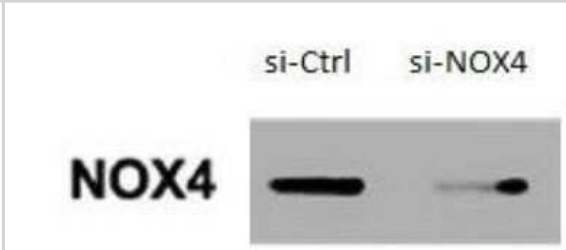
Immunohistochemistry-Paraffin: Nox4 Antibody - BSA Free [NB110-58849] - Analysis of a FFPE tissue section of mouse kidney using 1:200 dilution of NOX4 antibody (NB110-58849). The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.

Western Blot: Nox4 Antibody - BSA Free [NB110-58849] - Detection of NOX4 in HUVEC whole cell lysate. Lanes 1 and 2: serum-starved HUVEC lysate denatured at 95C for 5 minutes. Lanes 3: serum-starved HUVEC lysate denatured at room temperature for 10 minutes. Image from verified customer review.

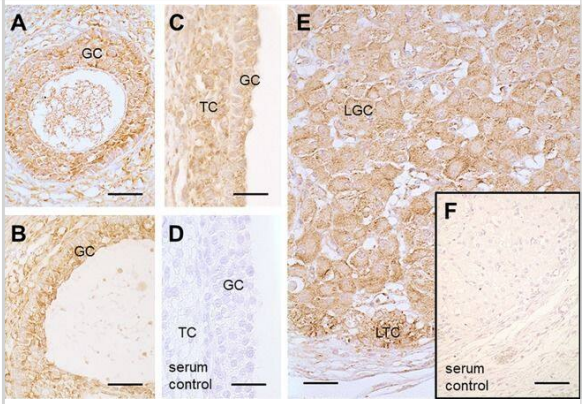


Immunocytochemistry/Immunofluorescence: Nox4 Antibody - BSA Free [NB110-58849] - Detection of NOX4 in mouse brain showing positive staining in neurons in the cortex. Image provided by Rachel Reith.

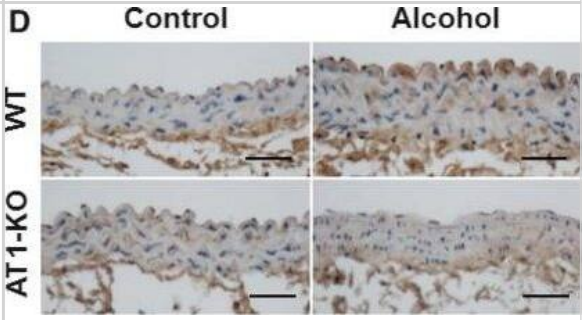


Immunohistochemistry: Nox4 Antibody - BSA Free [NB110-58849] - Detection of NOX4 in proximal convoluted tubules of the kidney using NB110-58849 at 5 ug/mL.	
Immunohistochemistry: Nox4 Antibody - BSA Free [NB110-58849] - AT1-KO mice are resistant to alcohol-induced oxidative stress. Oxidative stress was examined by immunohistochemical staining of NOX4 (D) expression was examined by immunohistochemical staining. Data are presented as means +/- SD. *, P < 0.05 vs corresponding control; #, P < 0.05 vs WT alcohol group. Bar = 50 uM. Image collected and cropped by CiteAb from the following publication (https://doi.wiley.com/10.1111/j.1582-4934.2012.01569.x), licensed under a CC-BY license.	
Knockdown Validated: Nox4 Antibody - BSA Free [NB110-58849] - DSC inhibits Nox4-mediated redox imbalance in BMDM. BMDM was treated with or without DSC (50 umol/L) or Nox4 siRNA. Representative bands and quantitative analysis of Nox4. Data shown are means +/- SEM of n = 8 in each group. *P < 0.05 vs Control cell (CTL), #P < 0.05 vs LPS-stimulated BMDM. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/32945118/) licensed under a CC-BY license.	
Knockdown Validated: Nox4 Antibody - BSA Free [NB110-58849] - Nox4 Antibody [NB110-58849] - Silencing of NOX4 in H4-II-C3 rat hepatoma cell line. Image from verified customer review.	

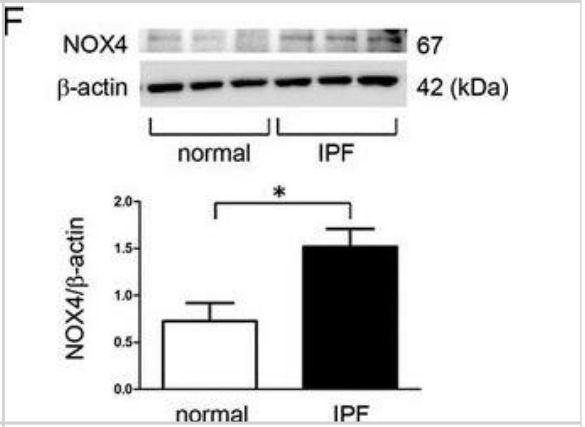
Presence of NOX4 in human ovarian tissue. Immunohistochemistry using human ovarian sections and an anti-NOX4 antibody from ProSci showed positive staining for NOX4 in granulosa (GC) and theca cells (TC) of a secondary follicle (A), of a small antral follicle (B), of a large antral follicle (C) as well as in luteinized GCs (LGC) and luteinized TCs (LTC) of the corpus luteum (E). Serum controls lacked first antibody (D and F). Scale bars: A–E = 30 µm, F = 50 µm.



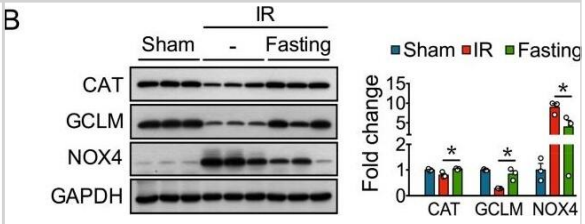
AT1-KO mice are resistant to alcohol-induced oxidative stress. Oxidative stress was examined by immunohistochemical staining of 3-NT (A) and 4 -HNE (B). NOX2 (C) and NOX4 (D) expression was examined by immunohistochemical staining. Data are presented as means ± SD (the animal number for each group is indicated in Figure 1). *, P < 0.05 vs corresponding control; #, P < 0.05 vs WT alcohol group. Bar = 50 µM.



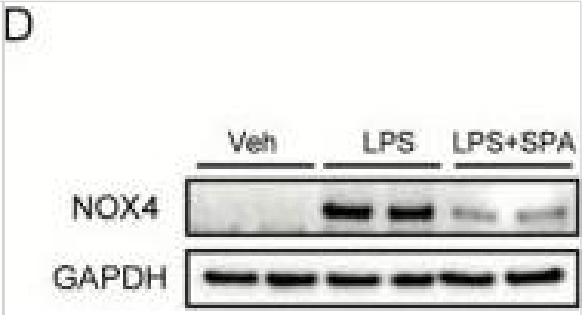
Western Blot: Nox4 Antibody - BSA Free [NB110-58849] - Effect of metformin on bleomycin-induced lung fibrosis development in mice. f WB using anti-NOX4, & anti-β-actin of cell lysates from normal LF (lane 1, 2, 3) & IPF LF (lane 4, 5, 6). Lower panel is average (±SEM) taken from 3patients shown as relative expression. Open bar is normal LF & filled bar is IPF LF. *p < 0.05 Image collected & cropped by CiteAb from following publication (http://respiratory-research.biomedcentral.com/articles/10.1186/s12931-016-0420-x), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



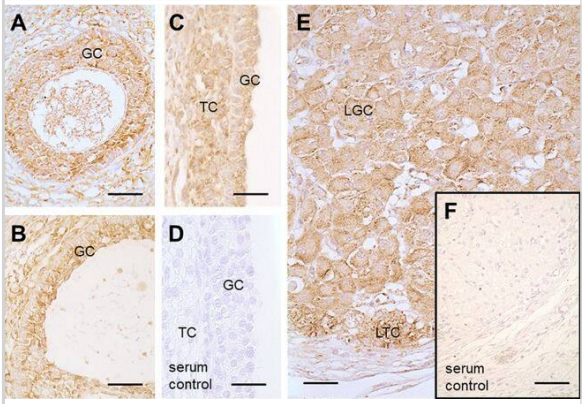
Western Blot: Nox4 Antibody - BSA Free [NB110-58849] - Fasting protects against oxidative stress resulting after two weeks of unilateral ischemia-reperfusion (IR) injury. (A) Representative kidney sections (10×) immunostained for 8-hydroxy-2′-deoxyguanosine (8-OHdG) in sham, IR, & IR + Fasting experimental groups at day 14 post-IR or sham surgery. (B) Immunoblots (left) & quantification (right) of catalase (CAT), glutamate-cysteine ligase modifier subunit (GCLM), & nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4) in the kidney cortex of rats from sham, IR, & IR + Fasting experimental groups. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Data are expressed as mean ± SEM. n = 3 animals per group. * p < 0.05. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31443530), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



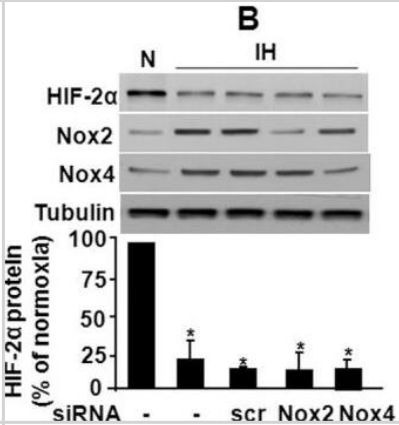
Western Blot: Nox4 Antibody - BSA Free [NB110-58849] - SPA0355 decreased renal oxidative stress & regulated levels of pro-oxidant & antioxidant enzymes in LPS-treated mice. (A) Representative images of IHC of 4-hydroxynonenal (4-HNE) in kidneys. Bar = 20 µm. (B) Percentage of 4-HNE-positive area per field. (C) Renal levels of malondialdehyde (MDA). (D) Representative images of Western blotting of nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4) & GAPDH. (E) The mRNA levels of manganese superoxide dismutase (MnSOD) & catalase. Results are from 8 mice per group (biological replicates) & 2 or 3 technical replicates per mouse. *** p < 0.001 vs. vehicle-treated mice (Veh). ###p <0.001 vs. LPS-injected mice (LPS). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32635491), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



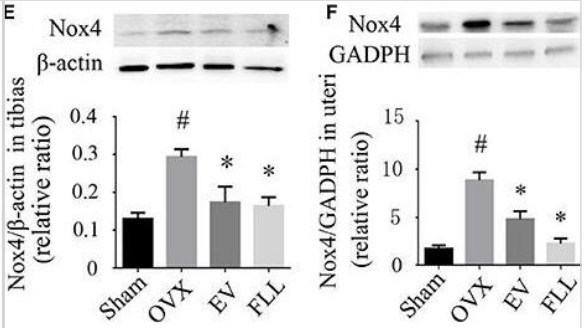
Immunohistochemistry: Nox4 Antibody - BSA Free [NB110-58849] - Presence of NOX4 in human ovarian tissue. Immunohistochemistry using human ovarian sections & an anti-NOX4 antibody from ProSci showed positive staining for NOX4 in granulosa (GC) & theca cells (TC) of a secondary follicle (A), of a small antral follicle (B), of a large antral follicle (C) as well as in luteinized GCs (LGC) & luteinized TCs (LTC) of the corpus luteum (E). Serum controls lacked first antibody (D & F). Scale bars: A–E = 30 µm, F = 50 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30837663), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



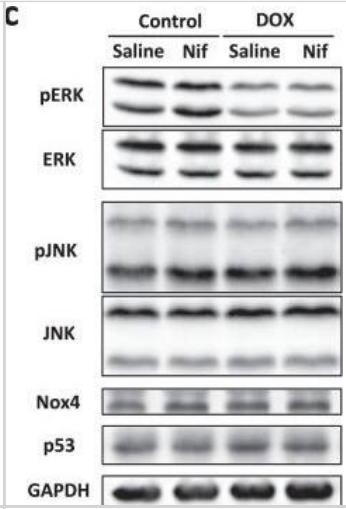
Western Blot: Nox4 Antibody - BSA Free [NB110-58849] - Role of NADPH oxidases in IH-induced HIF-2α degradation. A. Effect of NADPH oxidase (Nox) inhibitors Apocynin (Apo, 1 mM) & AEBSF (15 µM) on HIF-2α protein following exposure to IH. B. HIF-2α expression in PC12 cells transfected with Nox2 & Nox4 siRNA & exposed to normoxia (N) or IH. Tubulin expression was monitored as control for protein loading. Bottom panels of A & B represent average data of densitometric analysis of the immunoblots presented as mean ± S.E.M from three independent experiments. *p<0.05; n.s. not significant, p>0.05. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/24124516), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



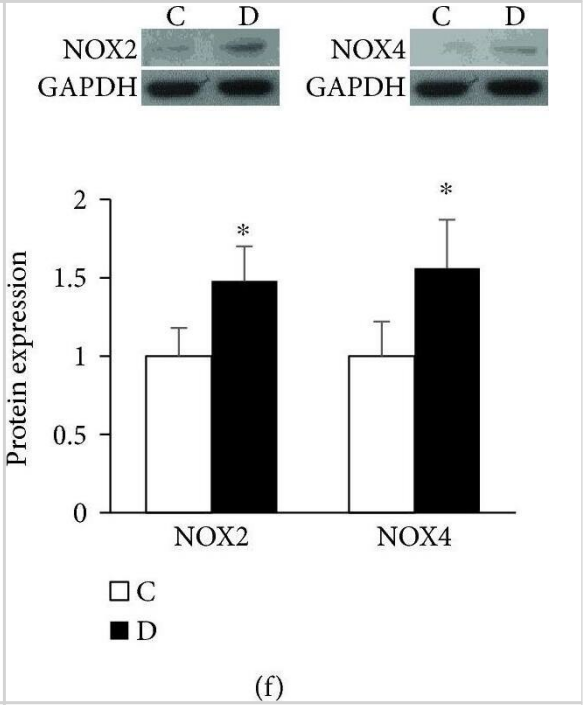
Western Blot: Nox4 Antibody - BSA Free [NB110-58849] - The representative images of immunohistochemical staining (A–D; sections were counterstained with hematoxylin; original magnification, × 20), & western blot assays (E,F) showed that FLL treatment decreased Nox4 expression in tibias & uteri of OVX rats (n = 9). In addition, FLL treatment also decreased cytochrome C (Cyto-C; G) & increased Bcl-2 expression (H) in the tibias of OVX rats (n = 9). Data are presented as mean ± SD. IOD denotes integrated optical density of interested areas. #p < 0.05 with Sham group, *p < 0.05 compared with OVX group. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28588482), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



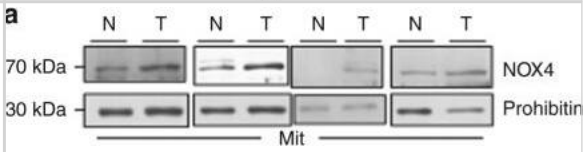
Western Blot: Nox4 Antibody - BSA Free [NB110-58849] - Blockade of LTCC suppressed CaMKII-NF-kB pathway in DOX-treated hearts. (a) Representative immunoblots & quantitative analysis of CaMKII, phosphorylated CaMKII, & GAPDH in DOX (3 doses of DOX at 6 mg/kg body weight every third day for 1 week) or control vehicle (phosphate-buffered saline: PBS) treated-C57B/6 J mouse hearts subjected to either nifedipine (Nif, 10 mg/day/day) or saline for 9 days (n = 5). (b) Representative immunoblots & quantitative analysis of NF-κB, phosphorylated NF-κB, cleaved caspase 3, & GAPDH in each group (n = 5). The experiment was conducted 3 times. (c–g) Representative immunoblotsa & quantitative analysis of ERK, phosphorylated ERK, JNK, phosphorylated JNK, Nox4, p53, & GAPDH in each group (n = 5). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31285514), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



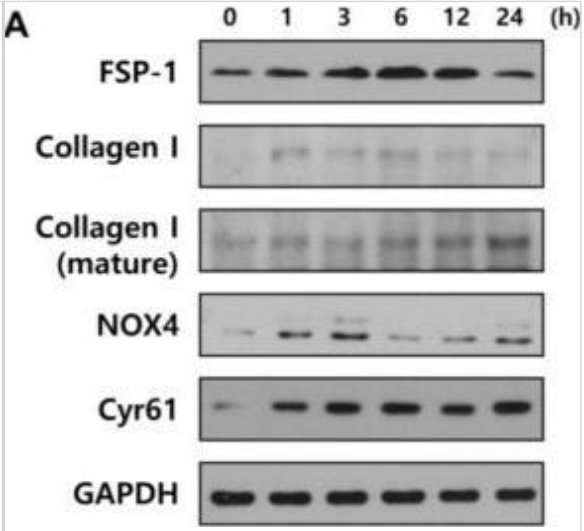
Western Blot: Nox4 Antibody - BSA Free [NB110-58849] - Diabetic atrophied muscles exhibited a state of heightened oxidative stress (HSOS). (a & b) Superoxide generation was measured in frozen muscle sections of control & diabetic using dihydroethidium-based confocal microscopic staining technique. (c & d) NADPH oxidase in a membrane fraction was assessed according to procedure involving the substrate NADPH & lucigenin chemiluminescence or the Amplex Red/horseradish peroxidase fluorescence-based assays. (e & f) Muscle NADPH oxidase-related isoforms including NOX2 & NOX4 were determined at the mRNA (e) & protein levels (f) using RT-PCR & Western blotting-based techniques. (g) Mitochondrial H2O2 generation at the steady state level & in the presence of added glutamate/malate substrates was measured using the Amplex Red/horseradish peroxidase fluorescence-based assay (g). Activities of complexes I (h) & III (i) of the electron transport chain were measured using spectrophotometric-based assay. Abbreviation: C: control; D: diabetic. Values are means ± SEM for at least 6 animals/group. □Significantly different from corresponding control values at P ≤ 0.05. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30510624), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



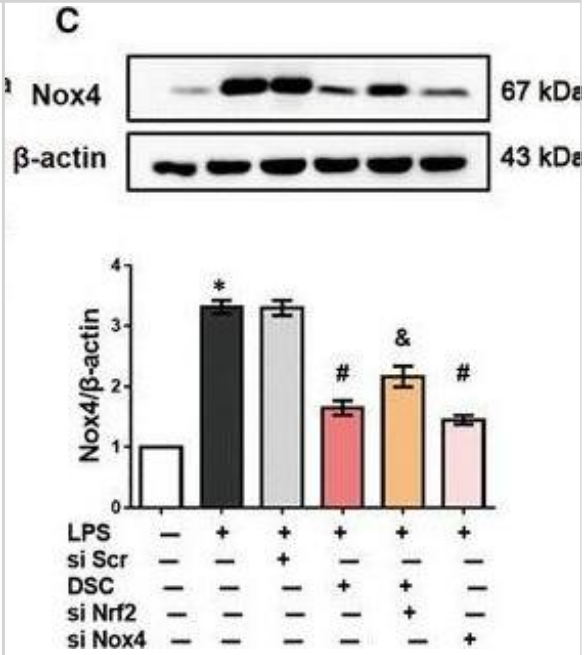
Western Blot: Nox4 Antibody - BSA Free [NB110-58849] - Characterization of NOX4 & PKM2 in human RCC tumors & adjacent tissue. a Mitochondrial fractions were prepared from human tumors (T) or uninvolved adjacent tissue (N). NOX4 expression was examined by western blot analysis. Prohibitin was probed as a mitochondrial marker & loading control. b Quantitation of NOX4 distribution in the mitochondrial fraction from a. The results are expressed as the means using one-way ANOVA with Tukey's post hoc test where ± S.E.M. *p < 0.05 compared to normal (N). c Mitochondria fractions were prepared from RCC tumors & NADPH-dependent superoxide generation was examined in the presence (+) or absence (−) of ATP. The results are from eight tumors & are expressed as the means using one-way ANOVA with Tukey's post hoc test where ±S.E.M. **p < 0.01 is compared to without (−) ATP. d PKM2 & PKM1 expression was examined by western blot analysis in lysates prepared from human tumors (T) or uninvolved adjacent tissue (N) from the same patient. Actin as loading control Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/29051480), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



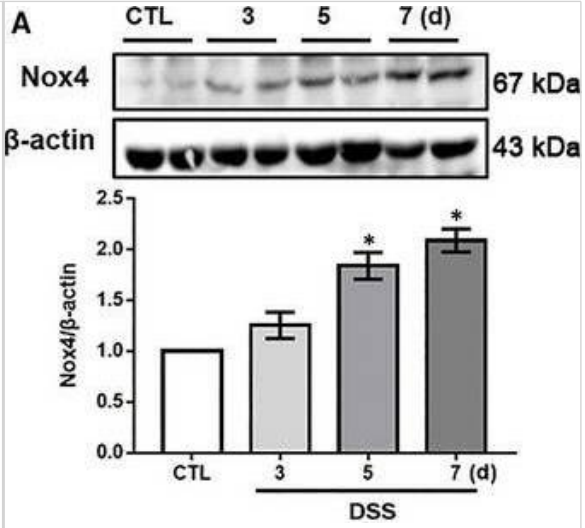
Western Blot: Nox4 Antibody - BSA Free [NB110-58849] - Fbs stimulated by BMM differentiate into myofibroblasts via the Cyr61/Nox4 pathway. (A) Western blotting for Fb differentiation, involving analysis of specific factors such as FSP-1, NOX2, NOX4, collagen-1, & Cyr61. The expression of these factors was normalized to that of GAPDH; (B) Cells were treated with BMM for 3 or 6 h or DPI (5 μM; NOX inhibitor) for 1 h or were co-treated with DPI & BMM. ROS production was measured using the DCF-DA assay & calculated as a percentage of the mean fluorescence intensity compared with that of the control. * $p < 0.05$ as compared to the control; # $p < 0.05$ as compared to the BMM (6 h)-treated group; (C) Cells were treated with BMM for 3 h or DPI (5 μM, NOX inhibitor) for 1 h or were co-treated with DPI & BMM. Cyr61 expression was normalized to that of GAPDH after western blotting. The values indicate intensities of protein expression with respect to that of the loading control; (D) Secretion of MMPs from BMM-treated-Fbs by using conditioned medium, followed by western blotting analysis. The expression levels were normalized to PonceauS, used as a loading control. *** $p < 0.001$ as compared to the control. Image collected & cropped by CiteAb from the following publication (http://www.mdpi.com/1422-0067/19/4/1164), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Nox4 Antibody - BSA Free [NB110-58849] - DSC restores redox balance in colonic tissues. A, Colitis was induced as described in Materials & Methods & treated with or without DSC (50 mg/kg). Representative bands & densitometry analysis of Nrf2 nuclear translocation & HO□1 expression in colonic tissues. Histone H3 & β□actin were used as loading control, respectively. B, Colitis was induced as described in Materials & Methods & treated with lentiviral Nox4 shRNA or lentiviral scrambled shRNA. Representative bands & densitometry analysis of Nrf2 nuclear translocation & HO□1 expression in colonic tissues. Histone H3 & β□actin were used as loading control, respectively. BMDM was stimulated with or without DSC (50 μmol/L) as described in Materials & Methods. C, Representative bands & densitometry analysis of Nox4. β□actin was used as loading control. D, Representative bands & densitometry analysis of Nrf2 nuclear translocation & cytoplasmic HO□1 expression in BMDM. β□actin or histone H3 was used as loading control. Data shown are means ± SEM of n = 8 in each group. *P < 0.05 vs Control cell or mice (CTL), #P < 0.05 vs DSS□treated mice or LPS□stimulated cells Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32945118), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Nox4 Antibody - BSA Free [NB110-58849] - DSC ameliorates Nox4 expression & ROS production. A, Colitis was induced as described in Materials & Methods, & the colonic tissues were collected as indicated periods. Representative bands & quantitative analysis of Nox4 in colonic tissues were shown. B□F, Colitis was induced as described in Materials & Methods & treated with or without DSC (50 mg/kg). B, Representative bands & quantitative analysis of Nox4 in colonic tissues. C, Representative images & quantitative analysis of ROS production by DHE staining in colonic tissues, scale bar = 100 μm. D, Quantitative analysis of GSH/GSSG ratio. E, Quantitative analysis of tissue H2O2 production. Colitis was induced as described in Materials & Methods & treated with or lentiviral Nox4 shRNA. F, Representative bands & quantitative analysis of Nox4 in colonic tissues. G, Representative images & quantitative analysis of colon length. H, Representative images of H&E staining & histological score. I, Representative bands & quantitative analysis of ZO□2 & claudin□1 expression in colonic tissues. β□actin was used as loading control. Data shown are means ± SEM of n = 8 in each group. *P < 0.05 vs Control (CTL), #P < 0.05 vs DSS□treated mice Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32945118), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Joung Eun Lee, Jung-Yeon Kim, Jaechan Leem, Claudio Ferrante, Julita Kulbacka Efficacy of Trametinib in Alleviating Cisplatin-Induced Acute Kidney Injury: Inhibition of Inflammation, Oxidative Stress, and Tubular Cell Death in a Mouse Model Molecules 2024-06-17 [PMID: 38930946]

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Christopher J Hanley, Massimiliano Mellone, Kirsty Ford, Steve M Thirdborough, Toby Mellows, Steven J Frampton, David M Smith, Elena Harden, Cedric Szyndralewiez, Marc Bullock, Fergus Noble, Karwan A Moutasim, Emma V King, Pandurangan Vijayanand, Alex H Mirnezami, Timothy J Underwood, Christian H Ottensmeier, Gareth J Thomas Targeting the Myofibroblastic Cancer-Associated Fibroblast Phenotype Through Inhibition of NOX4 JNCI Journal of the National Cancer Institute 2018-01-01 [PMID: 28922779]

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More publications at http://www.novusbio.com/NB110-58849

Procedures

Western Blot protocol for Nox4 Antibody (NB110-58849)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturer's instructions.

Immunohistochemistry-Paraffin Protocol for Nox4 Antibody (NB110-58849)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

Immunocytochemistry/Immunofluorescence protocol for Nox4 Antibody (NB110-58849)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

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NB110-58849PEP	Nox4 Antibody Blocking Peptide
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NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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