Product Datasheet

Nox4 Antibody
NB110-58849SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.

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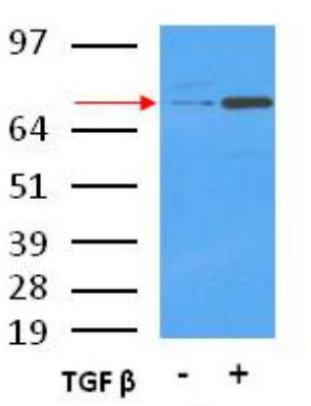
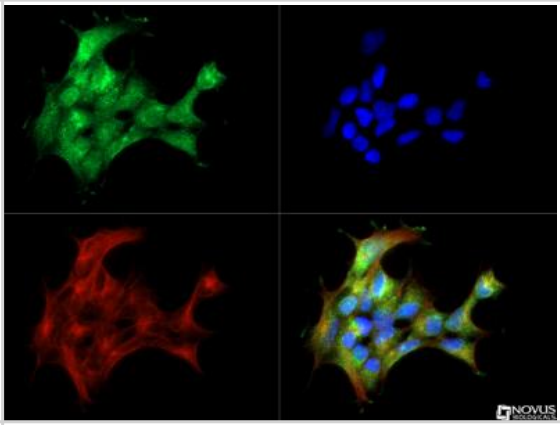
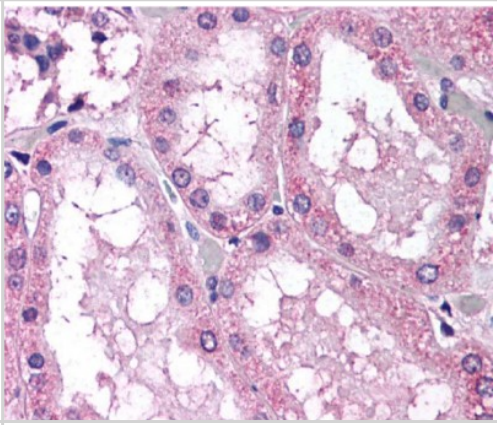
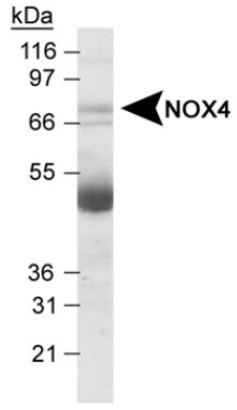
Nox4 Antibody

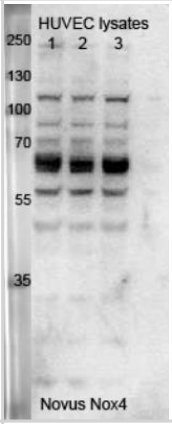
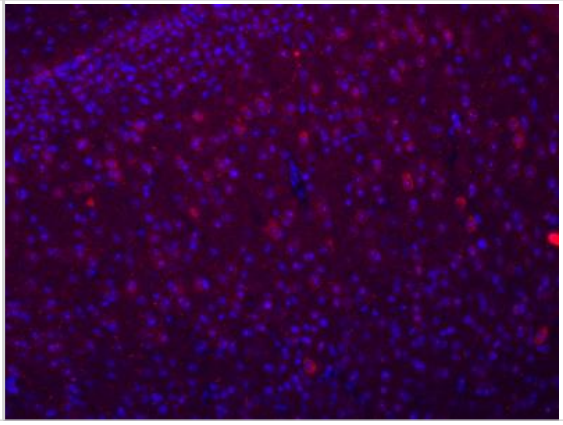
Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.1% Sodium Azide
Purity	Immunogen affinity purified
Buffer	Tris-citrate/phosphate, pH 7-8.

Product Description	
Host	Rabbit
Gene ID	50507
Gene Symbol	NOX4
Species	Human, Mouse, Rat, Bovine, Primate, Porcine, Rabbit, Sheep
Species Reactivity	Human, mouse, rat, bovine, sheep, primate and rabbit (see customer review). Procine 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, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Immunocytochemistry/Immunofluorescence 1:50-1:200, Immunohistochemistry 5 ug/ml, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin 5 ug/ml, Western Blot 2 ug/ml
Application Notes	This NOX4 antibody is useful for Western Blot, Immunocytochemistry/Immunofluorescence and Immunohistochemistry-paraffin embedded sections. Immunohistochemistry-Frozen was reported in scientific literature. 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 [NB110-58849] - Detection of NOX4 on IMR90 lysate. Image courtesy of Naomi Logsdon, University of Alabama at Birmingham.	
Immunocytochemistry/Immunofluorescence: NOX4 Antibody [NB110-58849] - NOX4 antibody was tested in Hek293 cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).	
Immunohistochemistry: NOX4 Antibody [NB110-58849] - Detection of NOX4 in proximal convoluted tubules of the kidney using NB110-58849 at 5ug/ml.	
Western Blot: NOX4 Antibody [NB110-58849] - Detection of NOX4 in human kidney lysates using NB110-58849 at 2.0 ug/ml. A non-specific band is often observed running at 50 kDa in tissue lysates which is believed to correspond to the human IgG heavy chain.	

Western Blot: NOX4 Antibody [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. Photo courtesy of product review by verified customer.	
Immunocytochemistry/Immunofluorescence: NOX4 Antibody [NB110-58849] - Detection of NOX4 in mouse brain showing positive staining in neurons in the cortex. Image provided by Rachel Reith.	

Publications

Yu C, Luo X, Duquette N et al. Knockdown of angiopoietin like-2 protects against angiotensin II-induced cerebral endothelial dysfunction in mice. Am. J. Physiol. Heart Circ. Physiol. 2014 Dec 19 [PMID: 25527773]

Sanders YY, Liu H, Liu G, Thannickal VJ. Epigenetic mechanisms regulate NADPH oxidase-4 expression in cellular senescence. Free Radic. Biol. Med. 2014 Dec 17 [PMID: 25526894] (WB, Human)

Milara J, Peiro T, Serrano A et al. Simvastatin Increases the Ability of Roflumilast N-oxide to Inhibit Cigarette Smoke-Induced Epithelial to Mesenchymal Transition in Well-differentiated Human Bronchial Epithelial Cells in vitro. COPD. 2014 Sep 10 [PMID: 25207459]

Kossmann S, Hu H, Steven S et al. Inflammatory Monocytes Determine Endothelial Nitric Oxide Synthase Uncoupling and Nitro-oxidative Stress Induced by Angiotensin II. J Biol Chem. 2014 Aug 20 [PMID: 25143378] (WB, Mouse)

Frazziano G, Al Ghouleh I, Baust J et al. Nox-derived ROS are acutely activated in pressure overload pulmonary hypertension: indications for a seminal role for mitochondrial Nox4. Am. J Physiol. Heart Circ. Physiol. 2014 Jan 15 [PMID: 24213612] (WB, Mouse)

Details:
NOX4 antibody used for WB in mitochondrial fractions from mouse's pulmonary artery banding experiments (Fig. 5) and right ventricular total lysate from NOX2 null mice (Fig. 6)

Terami N, Ogawa D, Tachibana H et al. Long-Term Treatment with the Sodium Glucose Cotransporter 2 Inhibitor, Dapagliflozin, Ameliorates Glucose Homeostasis and Diabetic Nephropathy in db/db Mice PLoS ONE. 2014 Jun 25 [PMID: 24960177] (IHC-P, Mouse)

Details:
Antibody used for IHC-P in mouse kidney tissue - deparaffinized sections immunostained with Nox4 antibody with 12 h incubation at 4C followed by HRP-conjugated goat anti-rabbit IgG antibody -DAB detection. NOX4 mainly localized in the interstitia of db/db mice with supressed levels in Dapagliflozin treated mice (Figure 5C).

Min HS, Kim JE, Lee MH et al. Dipeptidyl peptidase IV inhibitor protects against renal interstitial fibrosis in a mouse model of ureteral obstruction. Lab. Invest. 2014 Mar 31 [PMID: 24687121] (WB, Mouse)

Rozycki M, Lodyga M, Lam J et al. The fate of the primary cilium during myofibroblast transition. Mol. Biol. Cell 2014 Mar 1 [PMID: 24403605] (WB, Porcine)

Fandy TE, Jiemjit A, Thakar M et al. Decitabine Induces Delayed Reactive Oxygen Species (ROS) Accumulation in Leukemia Cells and Induces the Expression of ROS Generating Enzymes. Clin. Cancer Res. 2014 Mar 1 [PMID: 24423613] (WB, Human)

Decleves AE, Zolkipli Z, Satriano J et al. Regulation of lipid accumulation by AMK-activated kinase in high fat diet-induced kidney injury. Kidney Int 2013 Dec 4 [PMID: 24304883] (WB, Mouse)

Liu Y, Jia Z, Liu S et al. Combined losartan and nitro-oleic acid remarkably improves diabetic nephropathy in mice. Am J Physiol Renal Physiol 2013 Dec 1 [PMID: 23946292] (WB, Mouse)

Abe Y, Sakairi T, Beeson C, Kopp JB. TGF-beta1 stimulates mitochondrial oxidative phosphorylation and generation of reactive oxygen species in cultured mouse podocytes, mediated in part by the mTOR pathway. Am J Physiol Renal Physiol. 2013 Nov [PMID: 24049142] (WB, Mouse)

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

Procedures

Western blot Protocol specific for NOX4 antibody (NB110-58849)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer 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 manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunohistochemistry 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.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution 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 biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. 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 add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

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Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

For more information on our guarantee, please visit www.novusbio.com/guarantee.