Product Datasheet

Nox4 Antibody
NB110-58851

Unit Size: 0.1 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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Updated 7/11/2018 v.20.1

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**NB110-58851**
Nox4 Antibody

<table>
<thead>
<tr>
<th><strong>Product Information</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit Size</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Concentration</td>
<td>1.0 mg/ml</td>
</tr>
<tr>
<td>Storage</td>
<td>Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.02% Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG</td>
</tr>
<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td>Buffer</td>
<td>PBS (pH 7.4)</td>
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</tbody>
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**Product Description**

<table>
<thead>
<tr>
<th><strong>Host</strong></th>
<th>Rabbit</th>
</tr>
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<tbody>
<tr>
<td><strong>Gene ID</strong></td>
<td>50507</td>
</tr>
<tr>
<td><strong>Gene Symbol</strong></td>
<td>NOX4</td>
</tr>
<tr>
<td><strong>Species</strong></td>
<td>Human, Mouse, Rat, Porcine, Primate</td>
</tr>
<tr>
<td><strong>Reactivity Notes</strong></td>
<td>Human, mouse, rat, pig and primate.</td>
</tr>
<tr>
<td><strong>Immunogen</strong></td>
<td>A synthetic peptide made to a C-terminal region (within residues 500-578) of the human NOX4 protein sequence. [Swiss-Prot# Q9NPH5].</td>
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</tbody>
</table>

**Product Application Details**

<table>
<thead>
<tr>
<th><strong>Applications</strong></th>
<th>Western Blot, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recommended Dilutions</strong></td>
<td>Western Blot 2 - 4 ug/ml, Flow Cytometry, Immunohistochemistry 10 - 20 ug/ml, Immunocytochemistry/Immunofluorescence 10 - 20 ug/ml, Immunoprecipitation, Immunohistochemistry-Paraffin 10 - 20 ug/ml</td>
</tr>
<tr>
<td><strong>Application Notes</strong></td>
<td>This NOX4 antibody is useful for Western blot, ICC and Immunohistochemistry paraffin embedded sections. In Western Blot this NOX4 antibody recognizes bands at ~70kDa. Use in immunoprecipitation has reported in scientific literature (PMID 25062272)</td>
</tr>
</tbody>
</table>

**Images**

Western Blot: Nox4 Antibody [NB110-58851] - Analysis using the Biotin conjugate of NB110-58851. Detection of NOX4 in human kidney lysates using NB110-58851 at 0.5 ug/ml. Band observed at 31 kDa may represent reported splice isoform.
Immunocytochemistry/Immunofluorescence: Nox4 Antibody [NB110-58851] - HeLa cells were fixed in 10% buffered formalin for 10 min and permeabilized in 0.1% Triton X-100 in PBS for 10 min. Cells were incubated with antibodies to Nox4 (NB110-58851) and the ER marker Calreticulin (NBP1-47518), each at 20 ug/ml for 1 hour at room temperature. The coverslips were washed 3x in PBS and incubated with Alexa-Fluor 488 anti-rabbit secondary antibody. (green) and DyLight 550 anti-mouse secondary antibody. The merged image shows the co-localization of Nox4 and Calreticulin in the ER.


Western Blot: Nox4 Antibody [NB110-58851] - Whole cell protein from human HeLa, Hek293, HepG2, mouse Neuro2A and rat PC12 cells was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% BSA in TBST. The membrane was probed with 2.0 ug/ml anti-Nox4 in 1% BSA and detected with an anti-rabbit HRP secondary antibody using chemiluminescence. Nox4 is detected at approx. 70 kDa (arrowhead)

Immunocytochemistry/Immunofluorescence: Nox4 Antibody [NB110-58851] - HeLa cells were fixed in 10% buffered formalin for 10 min and permeabilized in 0.1% triton X-100 in PBS for 10 min. Cells were incubated with NB110-58851 at 20 ug/ml for 1 hour at room temperature, washed 3x in PBS and incubated with Alexa-Fluor 488 anti-rabbit secondary antibody. Nox4 (Green) was detected in the ER.

Publications


Feng C, Zhang Y, Yang M et al. Oxygen-sensing Nox4 generates genotoxic ROS to induce premature senescence of downloads.hindawi.com 2017 Aug 08 (WB, Rat)


Details:
This publication used the HRP conjugated form of this antibody (NB110-58851H)

More publications at http://www.novusbio.com/NB110-58851
Procedures

Protocol specific for Nox4 Antibody (NB110-58851)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 20-30 μg of total protein per lane.
2. Transfer proteins to PVDF according to the instructions provided by the manufacturer of the transfer apparatus.
3. Ponceau S stain the membrane for 1-2 minutes to confirm protein transfer. Rinse the blot in TBST to remove excess stain and mark the lane locations and molecular weight markers using a pencil.
4. Block the membrane in 5% BSA in TBST for 1 hour at room temperature.
5. Dilute the rabbit anti-NOX4 primary antibody (NB110-58851) in 1% BSA in TBST and incubate for 2 hours at room temperature.
6. Wash the membrane 3x for 10 min each in TBST.
7. Apply diluted rabbit-IgG HRP-conjugated secondary antibody prepared in 1% BSA TBST and incubate 1 hour at room temperature.
8. Wash the membrane 3x for 10 min each in TBST.
9. Apply the detection reagent of choice in accordance with the manufacturer’s instructions.

IHC-FFPE sections

I. Deparaffinization:
A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:
A. Place slides in peroxidase quenching solution: 15-30 minutes.
   To Prepare 200 ml of Quenching Solution:
   -Hydrogen Peroxide to 200 ml of Methanol.
   -Use within 4 hours of preparation
B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:
A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees C.
B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
D. Slowly add distilled water to further cool for 5 minutes.
E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:
A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
B. Flood slide with Wash Solution.
   Do not allow tissue sections to dry for the rest of the procedure.
C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes.
   Check development with microscope.
I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
M. Rinse slides in distilled water.

N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.

O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.

P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.

Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.

R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.

S. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:

- Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
- Prior to deparaffinization, heat slides overnight in a 60 degrees C oven.
- All steps in which Xylene is used should be performed in a fume hood.

For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
- For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
- 200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.
- 5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.
- Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1 1/2 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).
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 100% guarantee, please visit www.novusbio.com/guarantee

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