Product Datasheet

Nox4 Antibody
NB110-58849

Unit Size: 0.1 ml
Store at 4C. Do not freeze.

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# NB110-58849
Nox4 Antibody

## Product Information

<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</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. Do not freeze.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.05% 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</td>
</tr>
</tbody>
</table>

## Product Description

- **Host**: Rabbit
- **Gene ID**: 50507
- **Gene Symbol**: NOX4
- **Species**: Human, Mouse, Rat, Porcine, Bovine, Primate, Rabbit, Sheep
- **Reactivity Notes**: Human, mouse, rat, bovine, sheep, primate and rabbit (see customer review). 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

### Recommended Dilutions
- Western Blot 2 ug/ml, Immunohistochemistry 5 ug/ml, Immunocytochemistry/Immunofluorescence 1:50-1:200, Immunohistochemistry-Paraffin 5 ug/ml, Immunohistochemistry-Frozen, Immunoblotting

### 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. Use in Immunoblotting reported in scientific literature (PMID 28152080).
Western Blot: Nox4 Antibody [NB110-58849] - Silencing of NOX4 in H4-II-C3 rat hepatoma cell line. Image from verified customer review.

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


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

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.

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.

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).
### Publications


Dickson BJ, Gatie MI, Spice DM, Kelly GM. NOX1 and NOX4 are required for the differentiation of mouse F9 cells into extraembryonic endoderm. PLoS ONE. 2017 Feb 02 [PMID: 28152080] (IB, Mouse)

Chen L, Zhao M, Li J et al. Critical role of X-box binding protein 1 in NADPH oxidase 4-triggered cardiac hypertrophy is mediated by receptor interacting protein kinase 1. Cell Cycle. Dec 8 2016 12:00AM [PMID: 27929749]


**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 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.
4. Wash three times for 10 minutes.
5. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add 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 1:25,000 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.*
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.

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