Product Datasheet

iNOS Antibody - BSA Free

NB300-605

Unit Size: 200uL

Store at -20C. Avoid freeze-thaw cycles.

Reviews: 3  Publications: 118

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:

www.novusbio.com/NB300-605

Updated 12/20/2023 v.20.1

Earn rewards for product reviews and publications.

Submit a publication at www.novusbio.com/publications
Submit a review at www.novusbio.com/reviews/destination/NB300-605
# NB300-605

iNOS Antibody - BSA Free

## Product Information

<table>
<thead>
<tr>
<th>Feature</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit Size</td>
<td>200uL</td>
</tr>
<tr>
<td>Concentration</td>
<td>0.5 mg/ml</td>
</tr>
<tr>
<td>Storage</td>
<td>Store at -20C. 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>Affinity purified</td>
</tr>
<tr>
<td>Buffer</td>
<td>PBS</td>
</tr>
<tr>
<td>Target Molecular Weight</td>
<td>131 kDa</td>
</tr>
</tbody>
</table>

## Product Description

- **Host**: Rabbit
- **Gene ID**: 4843
- **Gene Symbol**: NOS2
- **Species**: Human, Mouse, Rat, Porcine

**Reactivity Notes**: Porcine reactivity reported in scientific literature (PMID: 31292486).

**Specificity/Sensitivity**: This antibody detects iNOS. It does not detect other NOS isoforms.

**Immunogen**: Synthetic peptide made to an internal portion of mouse iNOS (between amino acids 12-48) [UniProt P29477].

## Product Application Details

- **Applications**: Western Blot, Flow Cytometry, Immunoblotting, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, In vitro assay

**Recommended Dilutions**: Western Blot 1:200 - 1:800, Flow Cytometry reported in scientific literature (PMID 31536479), Immunohistochemistry 1:10 - 1:500, Immunocytochemistry/Immunofluorescence 1:50, Immunohistochemistry-Paraffin 1:20, Immunohistochemistry-Frozen reported in scientific literature (PMID 35005642), Immunoblotting, In vitro assay reported in scientific literature (PMID 27998907)

**Application Notes**: WB: Detects an approx. 135 kDa protein representing recombinant human iNOS and human iNOS from cytokine stimulated A549 cells. Also detects purified recombinant mouse iNOS, mouse iNOS from cytokine stimulated RAW 264.7 cells and cytokine stimulated rat fibroblast iNOS. However, the signals are not as strong as those seen with the human samples.
Western Blot: iNOS Antibody [NB300-605] - Analysis of iNOS was performed by loading 20 ug of RAW264 whole cell lysate untreated (left lane) or stimulated with LPS at 1 ug/mL for 16 hours (right lane) and 10 uL of PageRuler Plus Prestained Protein Ladder onto a 4-20% Tris-Glycine polyacrylamide gel. Proteins were transferred to a nitrocellulose membrane and blocked with 5% Milk in TBST for at least 1 hour. The membrane was probed with an iNOS Rabbit polyclonal antibody at a dilution of 1:1000 overnight at 4C on a rocking platform, washed in TBST, and probed with a Goat anti-Rabbit IgG (H+L) Secondary Antibody, HRP conjugate at a dilution of 1:1000 for 1 hour. Chemiluminescent detection was performed using SuperSignal West Pico.

Immunohistochemistry-Paraffin: iNOS Antibody [NB300-605] - Immunohistochemistry was performed on normal deparaffinized human Lung tissue.

Immunohistochemistry-Paraffin: iNOS Antibody [NB300-605] - Immunohistochemistry was performed on normal deparaffinized human Heart tissue.

Immunocytochemistry/Immunofluorescence: iNOS Antibody [NB300-605] - Analysis of iNOS in A549 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a iNOS polyclonal antibody at a dilution of 1:20 overnight at 4C, washed with PBS and incubated with a DyLight 488 conjugated secondary antibody. iNOS staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown.
Western Blot: iNOS Antibody [NB300-605] - iNOS in stimulated astrocytes. Western blot image submitted by a verified customer review.

Western Blot: iNOS Antibody [NB300-605] - LCA-induced oxidative stress in 4T1 breast cancer cells. The 4T1 cells were treated with LCA for 48 h, then the indicated measurements were performed. The level of iNOS protein was detected by western blotting (n = 3). Image collected and cropped by CiteAb from the following publication (https://www.mdpi.com/2072-6694/11/9/1255), licensed under a CC-BY license.

Immunocytochemistry/Immunofluorescence: iNOS Antibody [NB300-605] - Analysis of iNOS in NIH-3T3 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a iNOS polyclonal antibody at a dilution of 1:20 overnight at 4C, washed with PBS and incubated with a DyLight 488 conjugated secondary antibody. iNOS staining (green), F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown.
Indolepropionic acid (IPA) induced oxidative stress, cellular energy stress, and decreased the proportions of cancer stem cells. 500,000 cells/well 4T1 cells were treated with IPA in the concentrations indicated for 24 h; then, (A) lipid peroxidation was measured by TBARS assay, and (B) 4HNE expression was assessed by Western blotting (representative figure, \( n = 3 \)). In the same cells (C), the protein expression of NRF2 (at 68 kDa) and iNOS were determined by Western blotting (\( n = 3 \)), while (D) the mRNA expression of catalase (cat) was determined by RT-qPCR (\( n = 3 \)). (E) The expression of the indicated proteins (pACC, ACC, FOXO1, and PGC-1beta) were determined by Western blotting (\( n = 3 \), except for PGC-1beta, where \( n = 2 \)). (F) 100,000 cells/well 4T1 cells were treated with the indicated concentration of IPA for 24 h; then, the proportions of aldehyde dehydrogenase-positive cells were determined in Aldefluor assays using flow cytometry (\( n = 3 \)). For Western blots, a typical experiment was displayed. Fold data were log2 transformed to achieve normal distribution. Statistical significance was determined using the ANOVA test followed by Dunnett’s post-hoc test, except for panel F, where Student’s t-test was used. * and *** indicate statistically significant difference between control and treated samples at \( p < 0.05 \) and \( p < 0.001 \), respectively. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32854297), licensed under a CC-BY licence.

Inhibitory effect of MMPP on amyloidogenesis and STAT3 translocation in astrocytes and microglia cells. The expression of APP, BACE1 and C99 was detected by Western blotting using specific antibodies in astrocytes (a) and microglia cells (b). Each blot is representative of three experiments. The activity of beta-secretase was investigated using assay kit in astrocytes (c) and microglia cells (d). Values are presented as mean +/- S.D. of the three independent experiments performed in triplicate. 

\( \# p < 0.05 \) compared to control, 

\( *p < 0.05 \) compared to LPS.

Iba-1, COX-2, and iNOS proteins were detected by Western blotting using specific antibodies in astrocytes (e) and microglia cells (f). NO level was measured in astrocytes (g) and microglia cells (h). Activation of STAT3 was investigated using EMSA in astrocytes (i) microglial cells (j) were determined and the expression of STAT3 and phopho-STAT3 was also detected by Western blotting using specific antibodies (k), (l). For the cropped images, samples were run in the same gels under same experimental conditions and processed in parallel. Each band is representative for three experiments Image collected and cropped by CiteAb from the following publication (http://link.springer.com/10.1007/s12017-017-8469-3), licensed under a CC-BY licence.
Publications

Wu CY The effect of inducible nitric oxide synthase-ablation in pulmonary artery smooth muscle cells on cigarette smoke-induced pulmonary hypertension and emphysema development Thesis 2023-01-01 (IHC-P, Human, Mouse)


Raina K, Kandhari K, Jain AK et al. Stage-Specific Effect of Inositol Hexaphosphate on Cancer Stem Cell Pool during Growth and Progression of Prostate Tumorigenesis in TRAMP Model Cancers (Basel) 2022-08-30 [PMID: 36077751]


Kim JK, Yang HJ, Go Y. Quercus acuta Thunb. Suppresses LPS-Induced Neuroinflammation in BV2 Microglial Cells via Regulating MAPK/NF-?B and Nrf2/HO-1 Pathway Antioxidants (Basel) 2022-09-20 [PMID: 36290574]

Qiang P, Hao J, Yang F et al. Esaxerenone inhibits the macrophage-to-myofibroblast transition through mineralocorticoid receptor/TGF-?1 pathway in mice induced with aldosterone Frontiers in Immunology 2022-09-06 [PMID: 36148244] (ICC/IF)

Zhou Q, Lin L, Li H et al. Melatonin Reduces Neuroinflammation and Improves Axonal Hypomyelination by Modulating M1/M2 Microglia Polarization via JAK2-STAT3-Telomerase Pathway in Postnatal Rats Exposed to Lipopolysaccharide Molecular Neurobiology 2021-12-01 [PMID: 34585328] (EM, B/N)


Dravid AA, M Dhanabalan K, Agarwal S, Agarwal R. Resolvin D1-loaded nanoliposomes promote M2 macrophage polarization and are effective in the treatment of osteoarthritis Bioengineering & Translational Medicine 2022-05-01 [PMID: 35600665]


More publications at http://www.novusbio.com/NB300-605
Procedures

Immunohistochemistry-Paraffin Protocol for iNOS Antibody (NB300-605)
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.

Western Blot Protocol for iNOS Antibody (NB300-605)
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.
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

Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NB300-605

Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications