Product Datasheet

iNOS Antibody - BSA Free
NB300-605

Unit Size: 200uL

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

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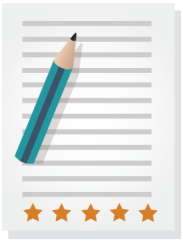
Updated 1/4/2024 v.20.1



NB300-605

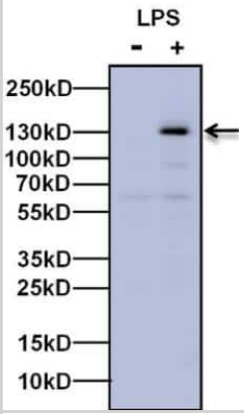
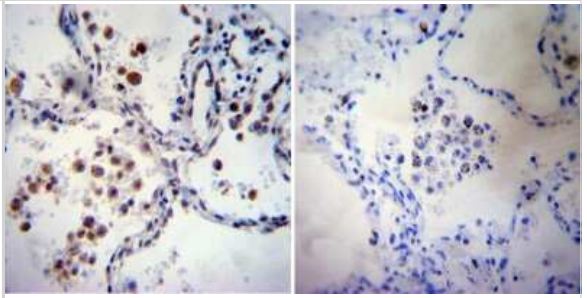
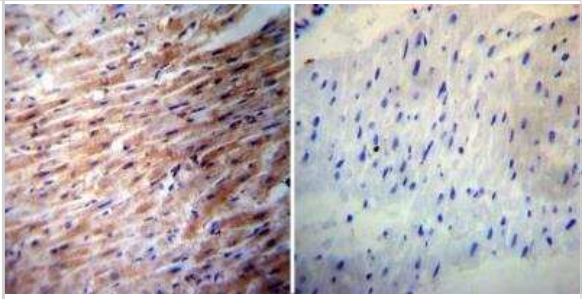
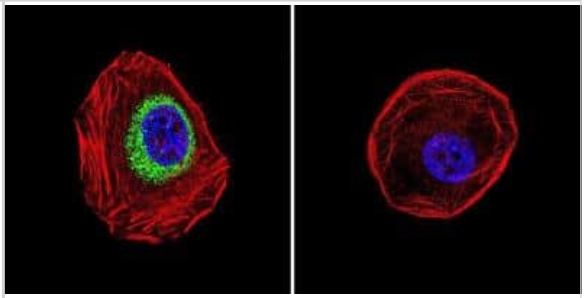
iNOS Antibody - BSA Free

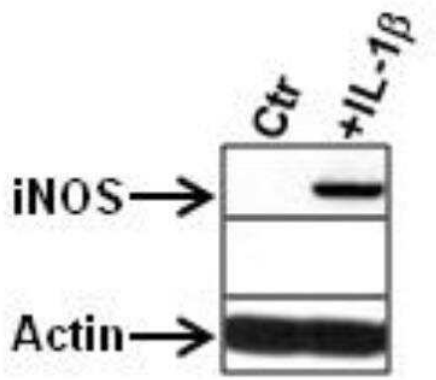
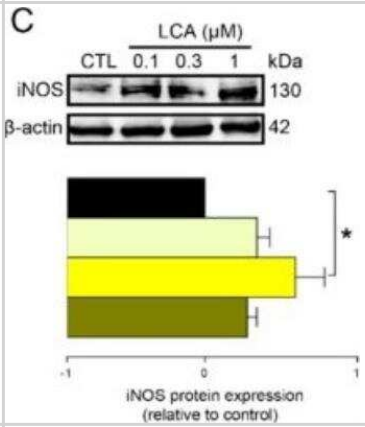
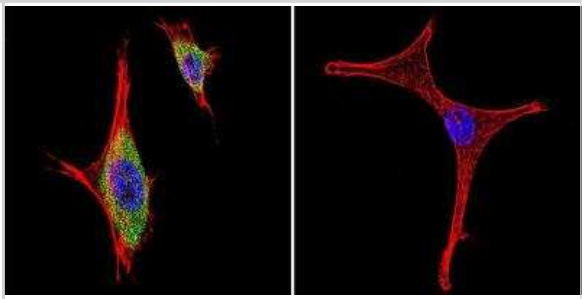
Product Information	
Unit Size	200uL
Concentration	0.5 mg/ml
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Affinity purified
Buffer	PBS
Target Molecular Weight	131 kDa

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

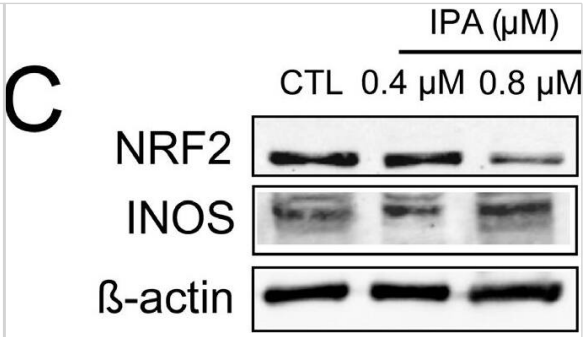
Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, In vitro assay
Recommended Dilutions	Western Blot 1:200 - 1:800, Flow Cytometry reported in 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.

Images

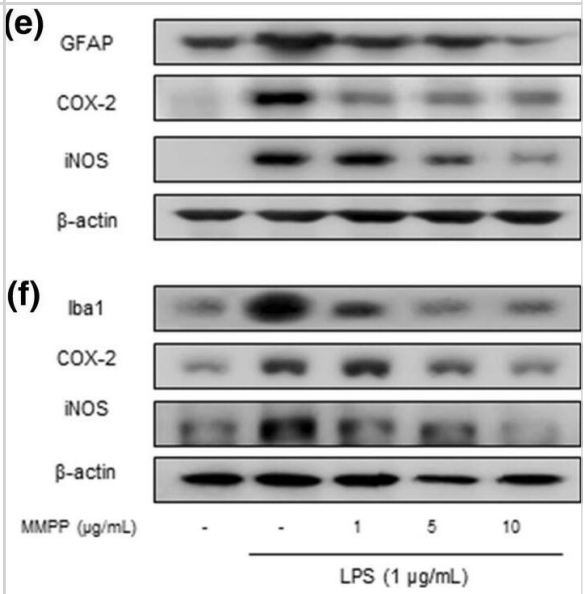
Western Blot: iNOS Antibody [NB300-605] - Analysis of iNOS was performed by loading 20 ug of RAW264 whole cell lysate untreated (left lane) or stimulated with LPS at 1 ug/mL for 16 hours (right lane) and 10 uL of PageRuler Plus Prestained Protein Ladder onto a 4-20% Tris-Glycine polyacrylamide gel. Proteins 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.	

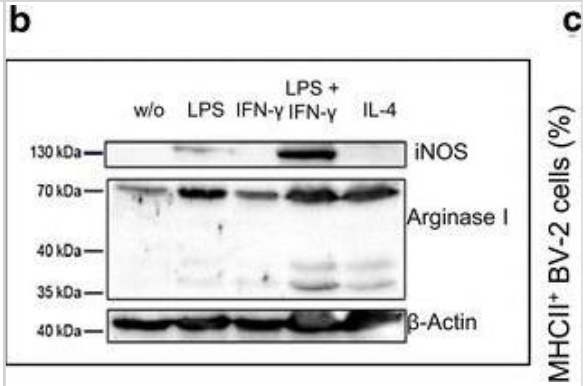
Indolepropionic acid (IPA) induced oxidative stress, cellular energy stress, and decreased the proportions of cancer stem cells. 500,000 cells/well 4T1 cells were treated with IPA in the concentrations indicated for 24 h; then, (A) lipid peroxidation was measured by TBARS assay, and (B) 4HNE expression 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 (https://link.springer.com/10.1007/s12017-017-8469-3), licensed under a CC-BY licence.



miR-27a/b prevent pUbS65 accumulation upon mitochondrial damage. a, b Overexpression of PINK1 increased pUbS65 accumulation in HeLa cells only when incubated with CCCP. 48 h post-transfection, cells were incubated with 10 μM CCCP for 2 h and pUbS65 levels were determined by Western blot (n = 3, two-way ANOVA). c, d Overexpression of miR-27a/b inhibited pUbS65 accumulation by CCCP in HeLa cells (n = 4, two-way ANOVA). e, f Inhibition of endogenous miR-27a/b increased pUbS65 accumulation by CCCP in HeLa cells (n = 4, two-way ANOVA). PINK1 levels were normalized to corresponding GAPDH level and quantified as a percentage of control. Values are mean ± SEM (n.s. = non-significant, **$p < 0.01$, ***$p < 0.001$) Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/27456084), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Hye Suk Baek, Victor Sukbong Hong, Hyunsu Kang, Sang-Jin Lee, Jin-Young Lee, Hyunju Kang, Seungik Jeong, Hyunho Jung, Jong Wook Park, Taeg Kyu Kwon, Chang-Nam Son, Sang Hyon Kim, Jinho Lee, Ki-Suk Kim, Shin Kim Anti-rheumatic property and physiological safety of KMU-11342 in in vitro and in vivo models. Inflammation research : official journal of the European Histamine Research Society ... [et al.] 2024-06-15 [PMID: 38879731]

Ines Köhler, Cecilia Bivik Eding, Nada-Katarina Kasic, Deepti Verma, Charlotta Enerbäck NOS2-derived low levels of NO drive psoriasis pathogenesis Cell Death & Disease 2024-06-26 [PMID: 38926337]

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S Kim, M Park, JY Kim, T Kim, JY Hwang, KS Ha, MH Won, S Ryoo, YG Kwon, YM Kim Circulating miRNAs Associated with Dysregulated Vascular and Trophoblast Function as Target-Based Diagnostic Biomarkers for Preeclampsia Cells, 2020-08-31;9(9):. 2020-08-31 [PMID: 32878300]

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Laura Jankó, Tünde Kovács, Miklós Laczik, Zsanett Sári, Gyula Ujlaki, Gréta Kis, Ibolya Horváth, Miklós Antal, László Vígh, Bálint L. Bálint, Karen Uray, Péter Bai, Ted M. Dawson, Oleh Khalimonchuk Silencing of Poly(ADP-Ribose) Polymerase-2 Induces Mitochondrial Reactive Species Production and Mitochondrial Fragmentation Cells 2021-06-04 [PMID: 34199944]

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SKH Chow, C Cui, KYK Cheng, YN Chim, J Wang, CHW Wong, KW Ng, RMY Wong, WH Cheung Acute Inflammatory Response in Osteoporotic Fracture Healing Augmented with Mechanical Stimulation is Regulated In Vivo through the p38-MAPK Pathway International Journal of Molecular Sciences, 2021-08-13;22(16):. 2021-08-13 [PMID: 34445423]

Chuanqi Cai, Sreenivasulu Kilari, Avishek K. Singh, Chenglei Zhao, Michael L. Simeon, Avanish Misra, Yiqing Li, Sanjay Misra Differences in Transforming Growth Factor□β1/BMP7 Signaling and Venous Fibrosis Contribute to Female Sex Differences in Arteriovenous Fistulas Journal of the American Heart Association: Cardiovascular and Cerebrovascular Disease 2020-08-06 [PMID: 32757791]

Haba D, Ohmiya T, Sekino M et al. Efficacy of wearable vibration dressings on full-thickness wound healing in a hyperglycemic rat model Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 2023-11-11 [PMID: 37950849] (IHC-P, Rat)

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)

Nin?evi? V, Zjali? M, Kolari? TO et al. Renoprotective Effect of Liraglutide Is Mediated via the Inhibition of TGF-Beta 1 in an LLC-PK1 Cell Model of Diabetic Nephropathy Current Issues in Molecular Biology 2022-02-25 [PMID: 35723295] (WB)

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.
13. Mount coverslips.

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.

Novus Biologicals USA
10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada
21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

Products Related to NB300-605

NBL1-13721	iNOS Overexpression Lysate
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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