

Product Datasheet

iNOS Antibody - BSA Free NB300-605

Unit Size: 200uL

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

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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

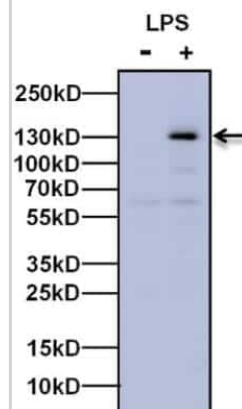
Product Description	
Description	Novus Biologicals Rabbit iNOS Antibody - BSA Free (NB300-605) is a polyclonal antibody validated for use in IHC, WB, Flow, ICC/IF and IP. Anti-iNOS Antibody: Cited in 132 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene 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, Immunohistochemistry-Paraffin, Flow Cytometry, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, 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.

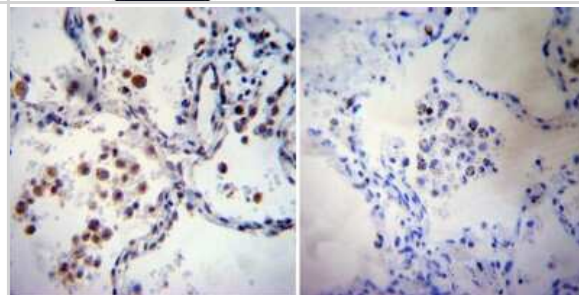


Images

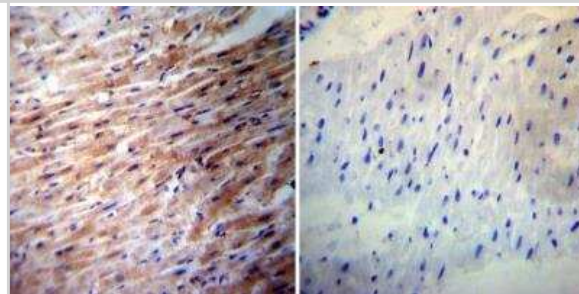
Western Blot: iNOS Antibody [NB300-605] - Analysis of iNOS was performed by loading 20 ug of RAW264 whole cell lysate untreated (left lane) or stimulated with LPS at 1 ug/mL for 16 hours (right lane) and 10 uL of PageRuler Plus Prestained Protein Ladder onto a 4-20% Tris-Glycine polyacrylamide gel. Proteins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



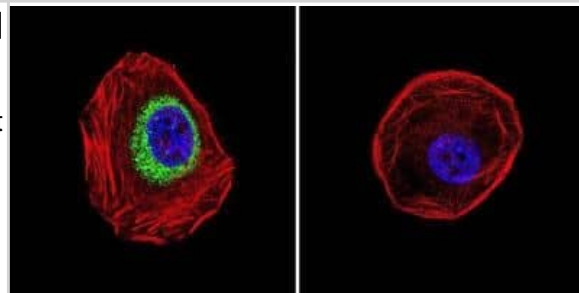
Immunohistochemistry-Paraffin: iNOS Antibody [NB300-605] - Immunohistochemistry was performed on normal deparaffinized human Lung tissue.



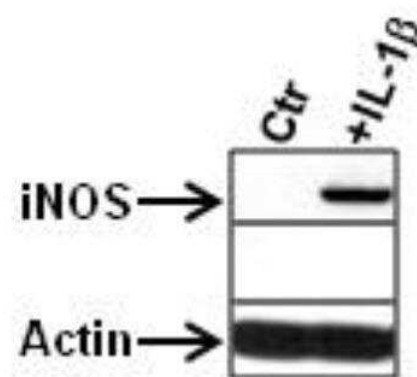
Immunohistochemistry-Paraffin: iNOS Antibody [NB300-605] - Immunohistochemistry was performed on normal deparaffinized human Heart tissue.



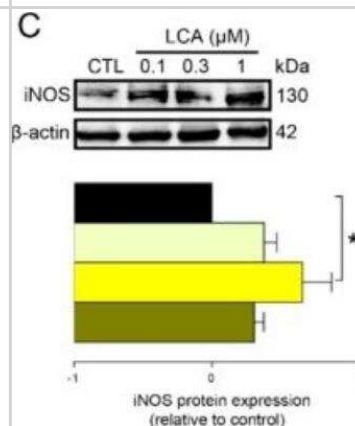
Immunocytochemistry/Immunofluorescence: iNOS Antibody [NB300-605] - Analysis of iNOS in A549 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a iNOS polyclonal antibody at a dilution of 1:20 overnight at 4C, washed with 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.



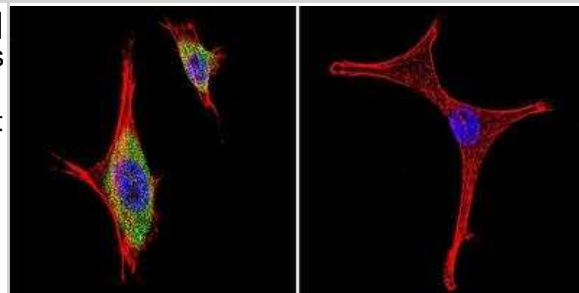
Western Blot: iNOS Antibody [NB300-605] - iNOS in stimulated astrocytes. Western blot image submitted by a verified customer review.



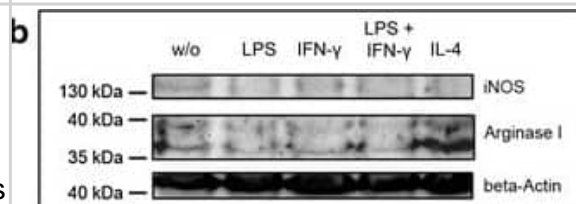
Western Blot: iNOS Antibody [NB300-605] - LCA-induced oxidative stress in 4T1 breast cancer cells. The 4T1 cells were treated with LCA for 48 h, then the indicated measurements were performed. The level of iNOS protein was detected by western blotting (n = 3). Image collected and cropped by CiteAb from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



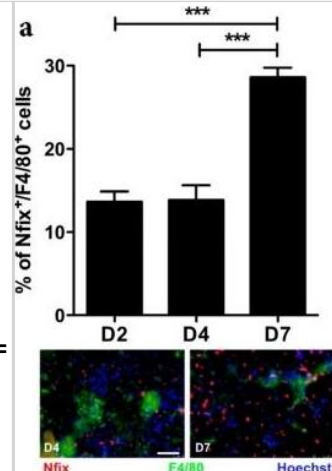
Immunocytochemistry/Immunofluorescence: iNOS Antibody [NB300-605] - Analysis of iNOS in NIH-3T3 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a iNOS polyclonal antibody at a dilution of 1:20 overnight at 4C, washed 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.



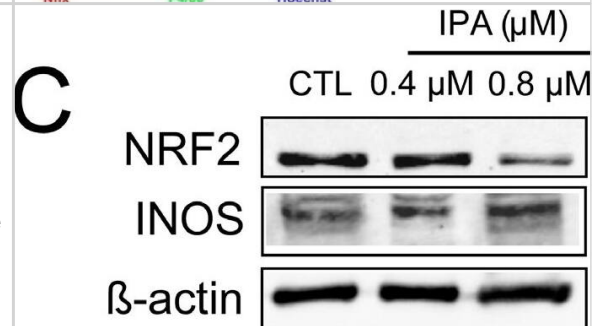
Western Blot: iNOS Antibody - BSA Free [NB300-605] - Stimulation of IMA 2.1 cells had no effect on relative survival after viral infection. IMA 2.1 cells were stimulated with LPS (10 μg/ml), IFN-γ, (10 ng/ml) LPS + IFN-γ, IL-4 (10 ng/ml), FGF (5 ng/ml) or FGF + LPS for 24 h in medium with 2% FBS. Polarization of cells was analyzed by Griess assay (a), Western blot (b) & FACS analysis by detection of MHCII+ cells (c). d MTT assay was performed to detect the relative percentage of VACV-mediated cell death in the presence of stimulating factors. e Viral replication was analyzed by standard plaque assay in the presence of stimulating factors. All experiments were performed in triplicate. Image collected & cropped by CiteAb from the following publication (<http://www.translational-medicine.com/content/13/1/216>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



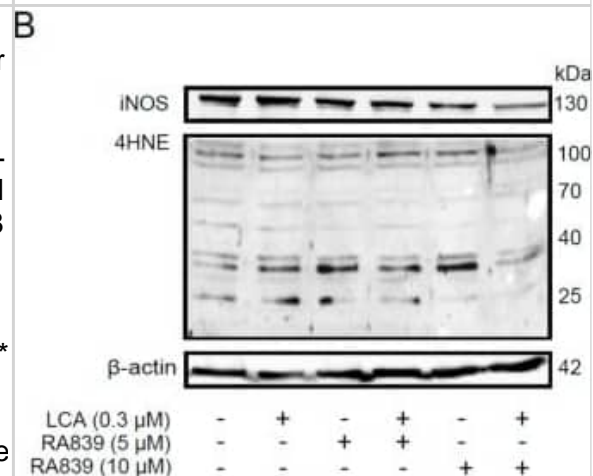
Immunocytochemistry/ Immunofluorescence: iNOS Antibody - BSA Free [NB300-605] - Nfix is mainly expressed by anti-inflammatory MPs. (a) Percentage of F4/80+ MPs positive for Nfix in Tibialis Anterior muscles (TA) of WT mice injected by CTX at D2, D4 & D7, post-injury. Immunostaining for F4/80 (green), Nfix (red) & DAPI (blue) at D4 & D7 after CTX injection; (b) Percentage of Ly6C+ & Ly6C- sorted MPs positive for Nfix in TA muscles of WT mice injected by CTX at D2, D4 & D7 post-injury; (c) Percentage of Nfix+ MPs after M1 & M2c polarization (with IFN γ & IL10, respectively). * $p < 0.05$; *** $p < 0.001$; for (b) * $p < 0.05$ Ly6C+ vs. Ly6C+ at D4 & D7; # $p < 0.05$ Ly6C- D7 vs. D2. Results are means \pm SEM of at least three independent experiments. Scale bar = 50 μ m. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32183151>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



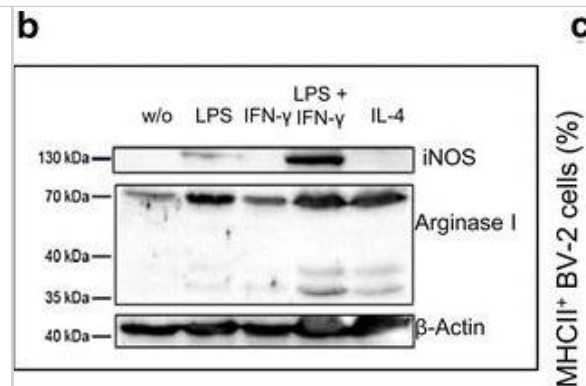
Western Blot: iNOS Antibody - BSA Free [NB300-605] - Indolepropionic acid (IPA) induced oxidative stress, cellular energy stress, & decreased the proportions of cancer stem cells. 500,000 cells/well 4T1 cells were treated with IPA in the concentrations indicated for 24 h; then, (A) lipid peroxidation was measured by TBARS assay, & (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) & 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, & PGC-1 β) were determined by Western blotting ($n = 3$, except for PGC-1 β , 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. * & *** indicate statistically significant difference between control & treated samples at $p < 0.05$ & $p < 0.001$, respectively. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/32854297>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



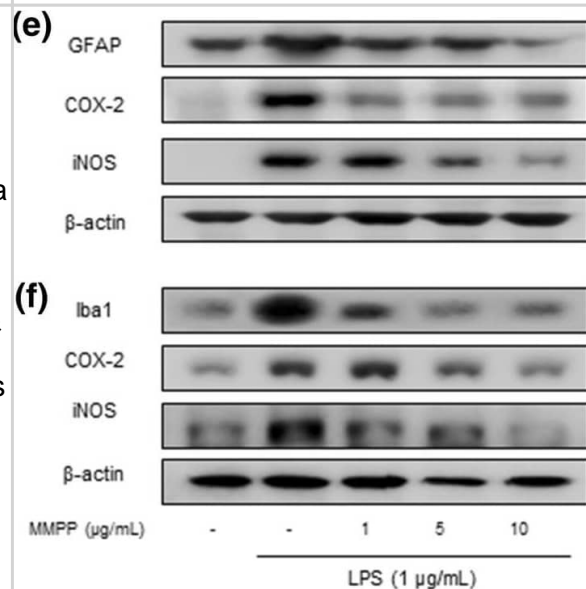
Western Blot: iNOS Antibody - BSA Free [NB300-605] - NRF2 activation modulated LCA-induced oxidative stress responses in 4T1 breast cancer cells. The 4T1 cells were treated with 0.3 μ M LCA & the NRF2 activator RA839 or tBHQ in the concentrations indicated for 48 h. Lipid peroxidation was determined by measuring (A) TBARS ($n = 4$) & (B,C) 4-HNE levels using western blotting ($n = 3$). (D) The 4T1 cells were treated with LCA (0.3 μ M) and/or GSH & NAC (both at 5 mM) antioxidants for 48 h, then total protein concentration was determined using the sulforhodamine B assay ($n = 3$). For statistical analysis ANOVA test was used followed by the Dunnett post-hoc test, where all groups were compared to the LCA-treated cohort. Data are plotted as mean \pm SEM. ** $p < 0.01$, LCA vs. LCA/NRF2-activator-treated groups (GSH, reduced glutathione; LCA, lithocholic acid; NAC, N-acetylcysteine; TBARS, thiobarbituric acid reactive substances; 4HNE, 4-hydroxynonenal). Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/31461945>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



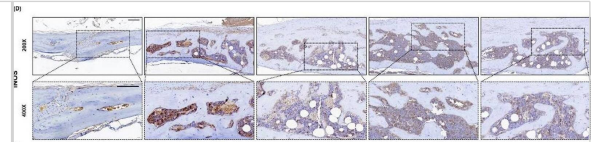
Western Blot: iNOS Antibody - BSA Free [NB300-605] - Impaired amount of virus progeny after stimulation of BV-2 cells with IFN- γ or IFN- γ & LPS. BV-2 cells were stimulated with LPS (10 $\mu\text{g/ml}$), IFN- γ , (10 ng/ml), LPS + IFN- γ or IL-4 (10 ng/ml) for 24 h in medium with 2% FBS. Polarization of cells was analyzed by Griess assay (a), Western blot (b) & by detection of the percentage of MHCII+ cells by FACS analysis (c). The amount of virus progeny (pfu/ml) was analyzed by standard plaque assay in the presence of stimulating factors & in normal growth medium in cells & supernatants (d). Statistical analysis was performed related to infection medium. To analyze the recovery of viral replication, normal growth medium was applied after infection of pre-stimulated cells. Standard plaque assay was performed to determine viral progeny in cells & supernatant after 24, 48, 72 & 96 hpi (e). To analyze the relative survival of the cells in the presence of stimulating factors a MTT assay was performed (f). The cellular density of stimulated cells relative to unstimulated (w/o) cells was detected via optical density measurement after 24 & 48 h of cultivation (g). Two-sided t test with unequal variances was used for statistics * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Active replication was defined as virus titer above the virus titer of the infection medium (red line). All experiments were performed in triplicate & repeated in an independent experiment. Image collected & cropped by CiteAb from the following publication (<http://www.translational-medicine.com/content/13/1/216>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: iNOS Antibody - BSA Free [NB300-605] - Inhibitory effect of MMPP on amyloidogenesis & STAT3 translocation in astrocytes & microglia cells. The expression of APP, BACE1 & C99 was detected by Western blotting using specific antibodies in astrocytes (a) & microglia cells (b). Each blot is representative of three experiments. The activity of β -secretase was investigated using assay kit in astrocytes (c) & microglia cells (d). Values are presented as mean \pm 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, & iNOS proteins were detected by Western blotting using specific antibodies in astrocytes (e) & microglia cells (f). NO level was measured in astrocytes (g) & microglia cells (h). Activation of STAT3 was investigated using EMSA in astrocytes (i) microglial cells (j) were determined & the expression of STAT3 & phospho-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 & processed in parallel. Each band is representative for three experiments Image collected & cropped by CiteAb from the following publication (<http://link.springer.com/10.1007/s12017-017-8469-3>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



TSA, PXD, or MGCD restore osteoimmune balance and reduce osteolytic activity. A Histological examination by H&E staining revealed differences in calvarial bone morphology between groups. Immunohistochemical staining demonstrated that treatment with TSA, PXD, or MGCD reduced osteoclast activity, as shown by decreased CTSK expression (B), suppressed M1 macrophage polarization indicated by reduced iNOS expression (D), enhanced M2 macrophage polarization indicated by increased ARG1 expression (F), and promoted osteogenesis as reflected by elevated RUNX2 expression (H). Quantification of CTSK- (C), iNOS- (E), ARG1- (G), and RUNX2- (I) positive cells. Data are presented as mean +/- SD (n = 6 per group). *P < 0.05, **P < 0.01, and ***P < 0.001. Scale bar = 100 μ m Image collected and cropped by CiteAb from the following open publication (<https://pubmed.ncbi.nlm.nih.gov/41174647>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Kang, DY;Bae, SW;Jang, KJ; Understanding the role of iron/heme metabolism in the anti-inflammatory effects of natural sulfur molecules against lipopolysaccharide-induced inflammation *Molecular medicine reports* 2025-07-01 [PMID: 40280116]

Latreille E, Mukkala A, Sanwal R et al. Phenotypic screening in influenza-infected zebrafish identifies Nrf2-mediated compound protective against ischemia-reperfusion injury *iScience* 2025-08-15 [PMID: 40734676]

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

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] (Western Blot)

Gallinat A, Mendieta G, Vilahur G et al. DJ-1 administration exerts cardioprotection in a mouse model of acute myocardial infarction *Frontiers in Pharmacology* 2022-09-23 [PMID: 36210822] (Western Blot)

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] (Western Blot)

Xie S, Pan J, Zhang Q et al. Japanese Encephalitis Virus (JEV) NS1' Enhances the Viral Infection of Dendritic Cells (DCs) and Macrophages in Pig Tonsils *Microbiology spectrum* 2022-06-22 [PMID: 35730942] (Western Blot)

Gu L, Feng C, Li M et al. Exosomal NOX1 promotes tumor-associated macrophage M2 polarization-mediated cancer progression by stimulating ROS production in cervical cancer: a preliminary study *Eur J Med Res* 2023-09-07 [PMID: 37679792] (Western Blot)

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] (Western Blot)

Choi JY, Hwang CJ, Lee HP et al. Inhibitory effect of ethanol extract of *Nannochloropsis oceanica* on lipopolysaccharide-induced neuroinflammation, oxidative stress, amyloidogenesis and memory impairment *Oncotarget* 2017-07-11 [PMID: 28489589] (Western Blot)

Prpar Mihevc S, Zakoek Pipan M, trbenc M et al. Nitrosative Stress in the Frontal Cortex From Dogs With Canine Cognitive Dysfunction *Frontiers in Veterinary Science* 2020-11-19 [PMID: 33330694] (Western Blot)

Fernandez-García S, Sancho-Balsells A, Longueville S et al. Astrocytic BDNF and TrkB regulate severity and neuronal activity in mouse models of temporal lobe epilepsy *Cell Death & Disease* 2020-06-01 [PMID: 32483154] (Western Blot)

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.



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Products Related to NB300-605

NBL1-13721	iNOS Overexpression Lysate
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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