Product Datasheet

LRRK2 Antibody - BSA Free
NB300-268SS

Unit Size: 0.025 ml

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

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Reviews: 1 Publications: 46

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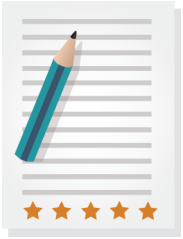
Updated 2/25/2025 v.20.1



NB300-268SS

LRRK2 Antibody - BSA Free

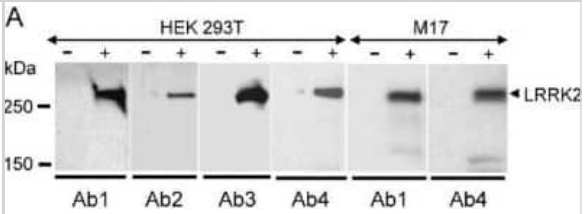
Product Information	
Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	286 kDa

Product Description	
Host	Rabbit
Gene ID	120892
Gene Symbol	LRRK2
Species	Human, Mouse, Bovine, C. elegans, Insect, Plant
Reactivity Notes	Human, Moth reactivity reported in scientific literature (PMID: 19824698). Plant reactivity reported in scientific literature (PMID: 27273569).
Immunogen	A C-terminal synthetic peptide made to the human LRRK2 protein sequence (between residues 2500-2527). [UniProt# Q5S007]

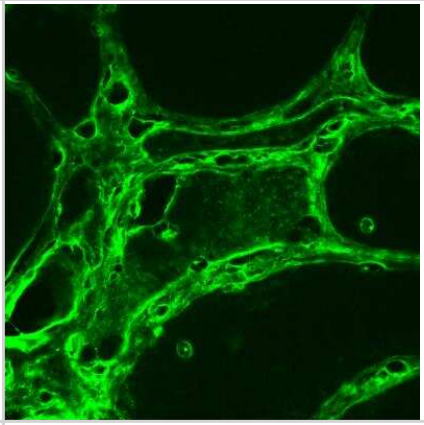
Product Application Details	
Applications	Western Blot, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Knockout Validated
Recommended Dilutions	Western Blot 1 - 2 ug/ml, Flow Cytometry 2.5 ug/ml, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 2 - 5 ug/ml, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen, Flow (Intracellular) 2.5 ug/ml, Knockout Validated
Application Notes	In Western blot, a band can be seen at ~286 kDa. We have also seen other bands with some lysates, but these bands have been blocked by the control peptide, suggesting that these bands are degradation products. IP has been done in an LRRK2 autophosphorylation kinase assay, IHC has been done on brain sections and ICC/IF has been done on transfected cell lines. Frozen sections using the LRRK2 antibody were from a customer review. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

Images

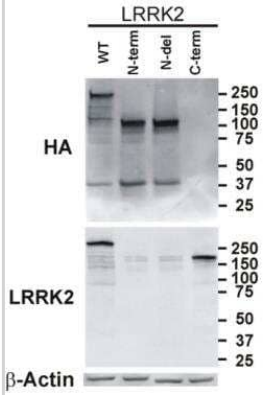
Western Blot: LRRK2 Antibody [NB300-268] - Western blotting of LRRK2. Recombinant LRRK2 (arrowhead) from transfected (+) HEK 293T and M17 cells was specifically recognized by all four LRRK2 antibodies (Ab1, Ab2, Ab3, and Ab4) used in this study. LRRK2 was not recognized in non-transfected cells (-). Image collected and cropped by CiteAb from the following publication (https://molecularneurodegeneration.biomedcentral.com/articles/10.1186/1750-1326-1-17), licensed under a CC-BY license.



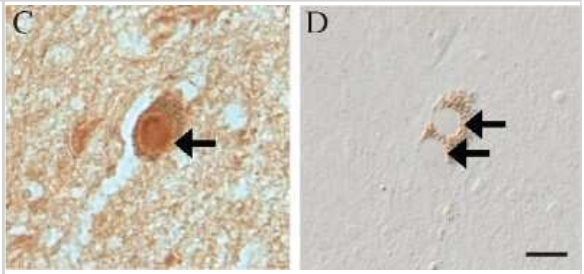
Immunohistochemistry-Frozen: LRRK2 Antibody [NB300-268] - Staining as described in PMID 24312256. Image from verified customer review.



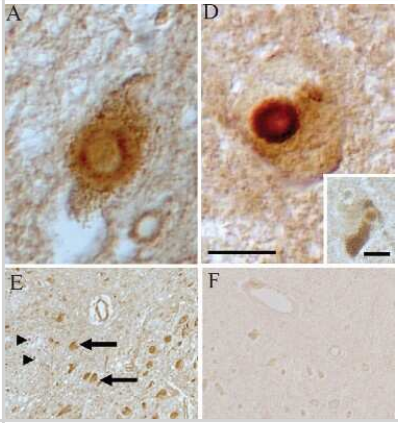
Western Blot: LRRK2 Antibody [NB300-268] - LRRK2 N-terminal sequences show increased aggregation. Western blots of cell lysates of SH-SY5Y cells transfected with indicated LRRK2 construct showed equal expression of the LRRK2 protein with anti-HA (top panel) or anti-C-terminal- (bottom panel) LRRK2 antibody. Equal lysate loading is indicated by Beta-actin loading control. Image collected and cropped by CiteAb from the following publication (//doi.org/10.1371/journal.pone.0045149) licensed under a CC-BY license.



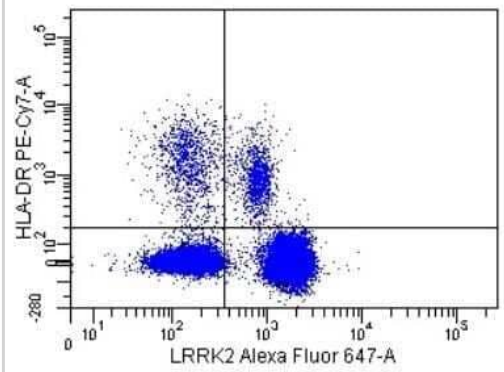
Immunohistochemistry-Paraffin: LRRK2 Antibody [NB300-268] - Specificity of immunostaining for LRRK2. Specificity of the antibodies used for immunocytochemistry was determined by performing adsorptions with the corresponding peptide sequences. Immunochemical localization of LBs (arrows) by Ab1 against LRRK2900-100 (left) was blocked using respective peptide antigens (right). Adjacent sections with LB within pigmented neurons from the substantia nigra of a case of PD were used. Scale bar: 20 um. Image collected and cropped by CiteAb from the following publication (https://molecularneurodegeneration.biomedcentral.com/articles/10.1186/1750-1326-1-17), licensed under a CC-BY license.



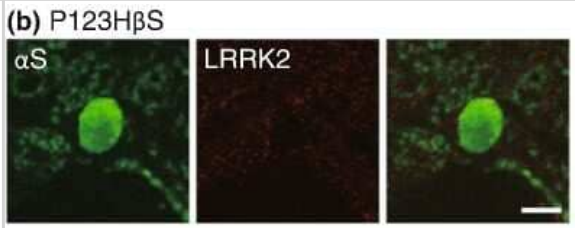
Immunohistochemistry-Paraffin: LRRK2 Antibody [NB300-268] - Immunocytochemistry of LRRK2 in PD using antibodies (Ab1, Ab4) raised against sequences corresponding to various regions shown on the schematic diagram of LRRK2 (red bars) were used on brainstem sections of PD. Scale bars: 10 um (top); E-L = 100 um (bottom). Image collected and cropped by CiteAb from the following publication (https://molecularneurodegeneration.biomedcentral.com/articles/10.1186/1750-1326-1-17), licensed under a CC-BY license.



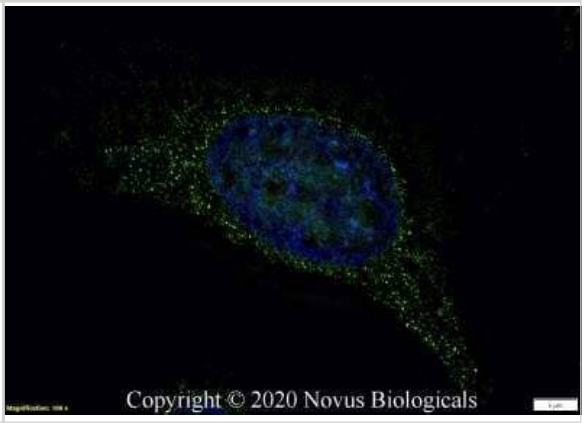
Flow Cytometry: LRRK2 Antibody [NB300-268] - Analysis using the Alexa Fluor 647 conjugate of NB300-268. Staining of LRRK2 in normal human peripheral blood cells (anticoagulated Lithium-Heparin) using anti-LRRK2 antibody conjugated with AF647. The primary antibody was used at a dilution of 1:200 and incubated for 20 min at room temperature. Image from verified customer review.



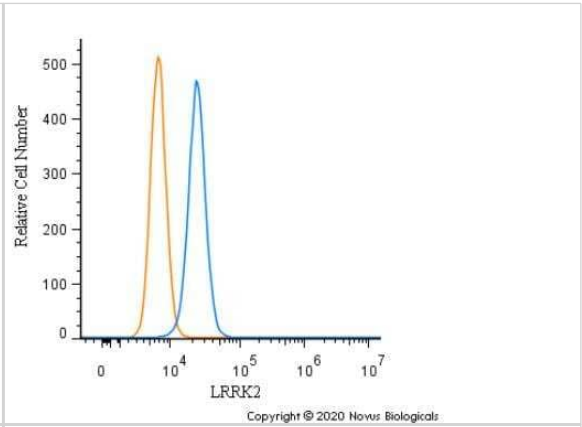
Immunohistochemistry-Paraffin: LRRK2 Antibody [NB300-268] - LRRK2 accumulates in globules in alphaS tg mice. Triple immunofluorescence for alphaS, LRRK2 and Rab5B for basal ganglia in alphaS tg mice. LRRK2 and Rab5B were colocalized in axon terminal (arrow), but were not colocalized in the alphaS-globule (arrowhead) Scale bar = 10 um for all panels. Image collected and cropped by CiteAb from the following publication (https://molecularbrain.biomedcentral.com/articles/10.1186/1756-6606-5-34), licensed under a CC-BY license.



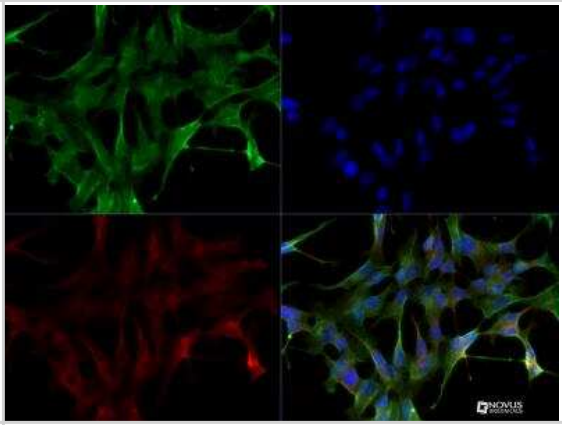
Immunocytochemistry/Immunofluorescence: LRRK2 Antibody [NB300-268] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-LRRK2 Antibody NB300-268 at 2 ug/ml overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.



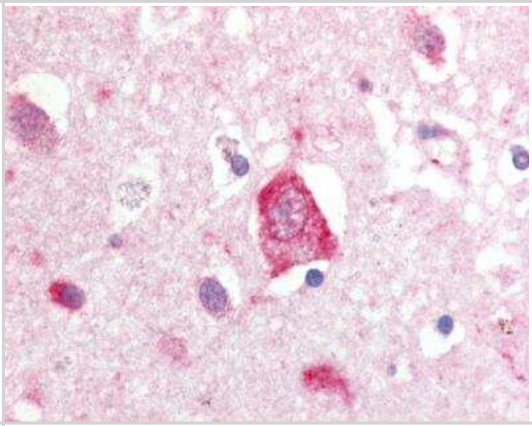
Flow Cytometry: LRRK2 Antibody [NB300-268] - An intracellular stain was performed on U2OS cells with LRRK2 Antibody NB300-268 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).



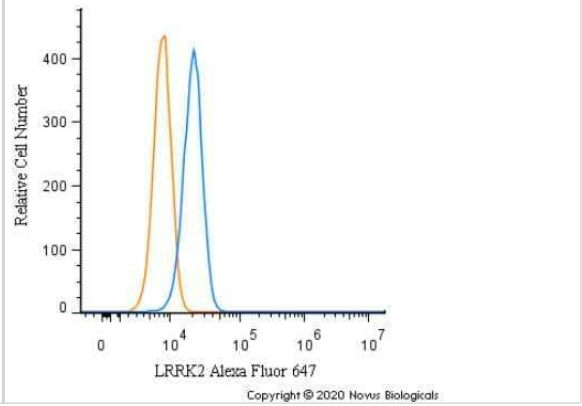
Immunocytochemistry/Immunofluorescence: LRRK2 Antibody [NB300-268] - LRRK2 antibody was tested in SH-SY-5Y cells at a 1:200 dilution against DyLight 488 (Green). Alpha tubulin and nuclei were counterstained against DyLight 568 (Red), and DAPI (Blue), respectively.

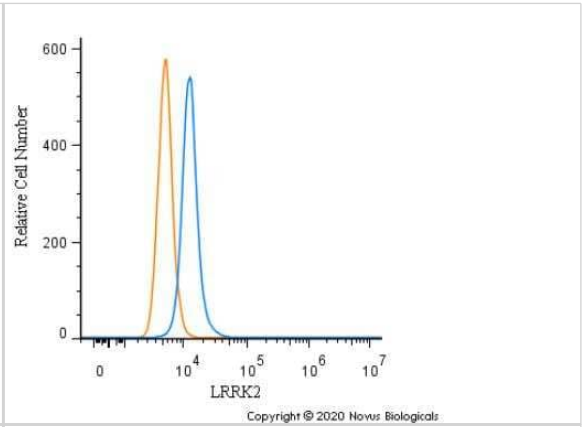
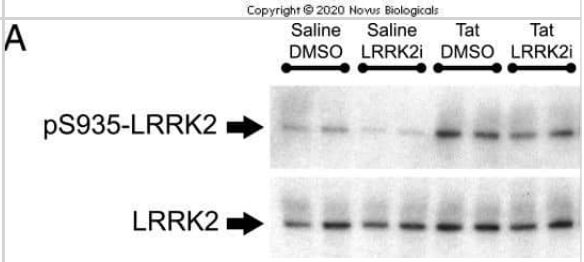
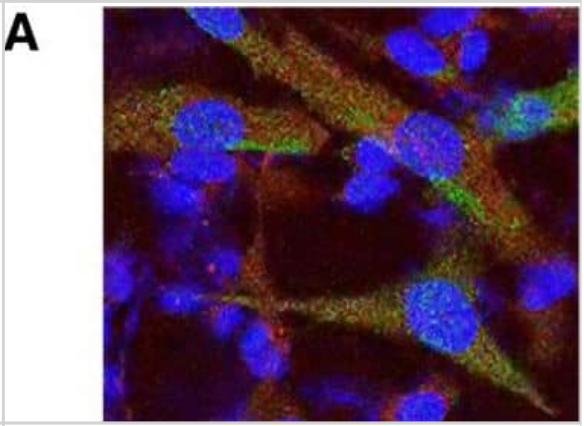
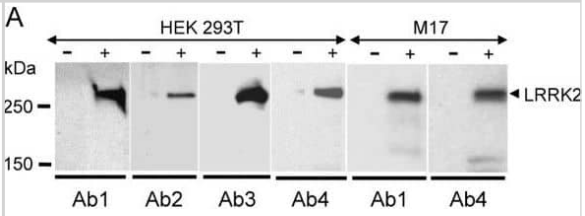


Immunohistochemistry-Paraffin: LRRK2 Antibody [NB300-268] - Staining of human brain, Putamen, Neurons and Glia using a 60X magnification.

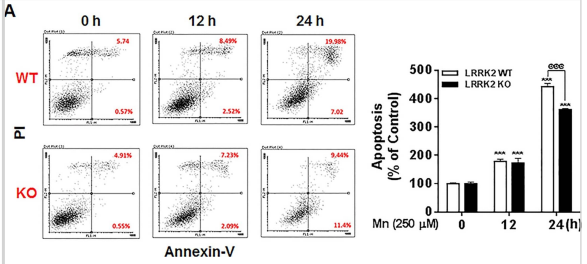


Flow Cytometry: LRRK2 Antibody [NB300-268] - An intracellular stain was performed on U2OS cells with LRRK Antibody NB300-268AF647 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 647.



Flow Cytometry: LRRK2 Antibody [NB300-268] - An intracellular stain was performed on Neuro2a cells with LRRK2 Antibody NB300-268 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 2.5 ug/mL for 30 minutes at room temperature, followed by Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (SA5-10033, Thermo Fisher).	
LRRK2 kinase inhibition blocks Tat-induced S935 phosphorylation and inflammatory cytokine expression in BV-2 cells. (A) Western blot depicting pS935-LRRK2 and total LRRK2 protein levels 12 hours after saline or Tat-treatment ± LRRK2 kinase inhibition (LRRK2i).	
The interaction and phosphorylation of LRRK2 and LC1. (A) Cellular co-localisation of endogenous LRRK2 and MAP1B (LC1). SKNSH cells were labelled with LRRK2 (green) and LC1 (red) antibodies and signals were detected by immunofluorescence. Detailed figure in Additional file 1.	
Western Blot: LRRK2 Antibody - BSA Free [NB300-268] - Western blotting of LRRK2. A. Recombinant LRRK2 (arrowhead) from transfected (+) HEK 293T & M17 cells was specifically recognized by all four LRRK2 antibodies (Ab1, Ab2, Ab3, & Ab4) used in this study. LRRK2 was not recognized in non-transfected cells (-). B. Recombinant LRRK2 (arrowhead) from transfected M17 cells is detected by anti-LRRK2 Ab4 (lane 1), but not after absorption of the antibody with its peptide antigen (lane 2). Brain LRRK2 was recognized by anti-LRRK2 Ab4 in two controls (lanes 3 & 4) & two PD cases (lanes 5 & 6). Cell lysates (10 µg protein) & brain homogenates (100 µg protein) were prepared & loaded on 6% SDS-PAGE gels for Western blot analysis using anti-LRRK2 antibodies Ab1–Ab4 in (A) & Ab4 in (B). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/17137507), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Flow Cytometry: LRRK2 Antibody - BSA Free [NB300-268] - Deletion of LRRK2 attenuates Mn-induced apoptosis & cell death in RAW 264.7 cells.(A) LRRK2 WT or KO RAW 264.7 cells were treated with Mn (250 µM) for the designated times, followed by flow cytometry analysis to determine Mn-induced apoptosis. Both early & late apoptotic cells (Q2 & Q3) were measured. (B) At the end of Mn exposure, cell viability was determined by MTT assay. @@@, p < 0.001; ###, p < 0.001; ***, p < 0.001 compared to the control (one-way ANOVA followed by Tukey's post hoc test; n = 3, for apoptosis assay; n = 6, for MTT assay). The data shown are representative of 3 independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30645642), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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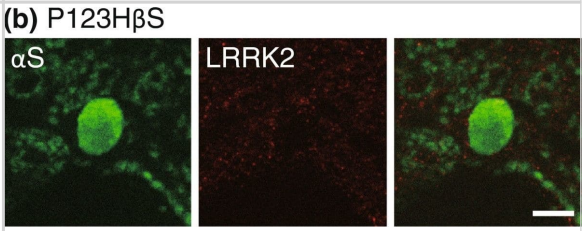
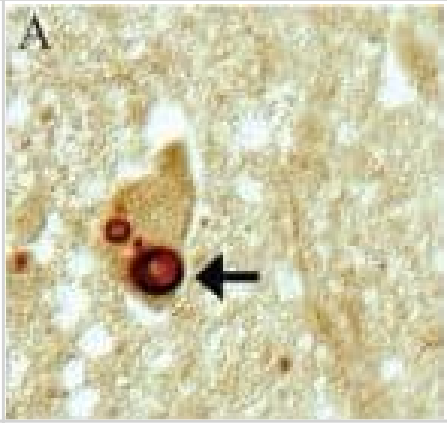
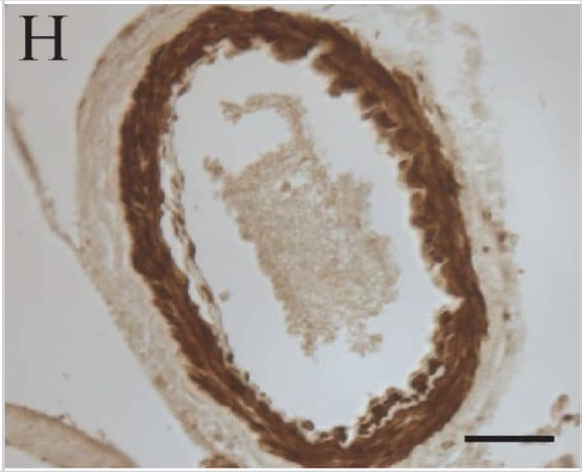


Immunohistochemistry-Paraffin: LRRK2 Antibody - BSA Free [NB300-268] - Specificity of immunostaining for LRRK2. Specificity of the antibodies used for immunocytochemistry was determined by performing adsorptions with the corresponding peptide sequences. Immunochemical localization of LBs (arrows) by Ab4 against LRRK22500–2527 (A) & Ab1 against LRRK2900–100 (C) was blocked using respective peptide antigens (B & D). Adjacent sections with LB within pigmented neurons from the substantia nigra of a case of PD were used. Scale bar: 20 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/17137507), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Immunohistochemistry-Paraffin: LRRK2 Antibody - BSA Free [NB300-268] - Immunocytochemistry of LRRK2 in DLB. Cortical LBs (arrows) in DLB were positive for LRRK2 using both Ab4 against LRRK22500–2527 (A), & Ab1 against LRRK2900–100 (B). Neuronal cytoplasm (C) was also strongly labelled throughout the cortex by Ab4 & with a consistently more granular pattern by Ab1 (inset in C) in cases of DLB. Control cases display similar, though less intense neuronal labeling (D). Biochemically purified cortical LBs were strongly positive for staining by Ab4 (E) as well as by anti-α-synuclein (F), while they were unstained when omitting primary antibody (G). Large vessels (H) were also intensely labelled by Ab4. Scale bars: A, B = 25 µm; C, D = 100 µm; E-G = 25 µm; H = 50 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/17137507), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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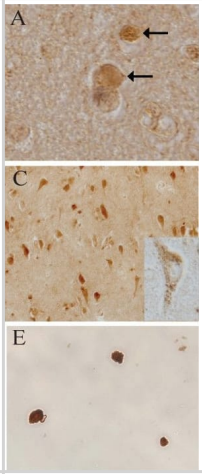

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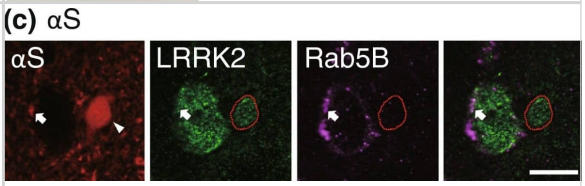

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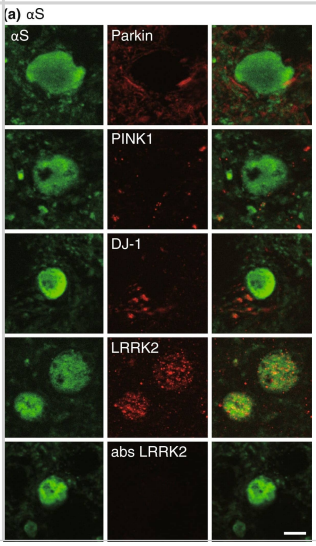
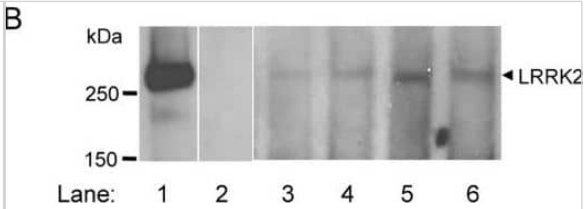
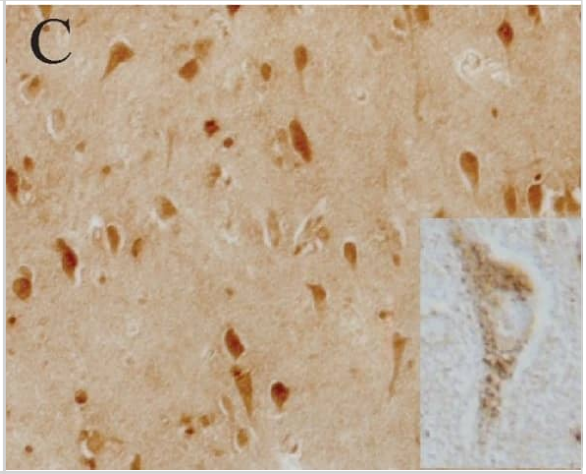


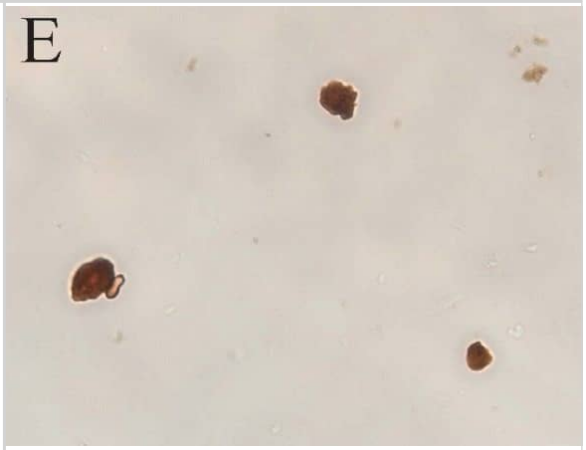
Western Blot: LRRK2 Antibody - BSA Free [NB300-268] - Western blotting of LRRK2. A. Recombinant LRRK2 (arrowhead) from transfected (+) HEK 293T & M17 cells was specifically recognized by all four LRRK2 antibodies (Ab1, Ab2, Ab3, & Ab4) used in this study. LRRK2 was not recognized in non-transfected cells (-). B. Recombinant LRRK2 (arrowhead) from transfected M17 cells is detected by anti-LRRK2 Ab4 (lane 1), but not after absorption of the antibody with its peptide antigen (lane 2). Brain LRRK2 was recognized by anti-LRRK2 Ab4 in two controls (lanes 3 & 4) & two PD cases (lanes 5 & 6). Cell lysates (10 µg protein) & brain homogenates (100 µg protein) were prepared & loaded on 6% SDS-PAGE gels for Western blot analysis using anti-LRRK2 antibodies Ab1–Ab4 in (A) & Ab4 in (B). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/17137507), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



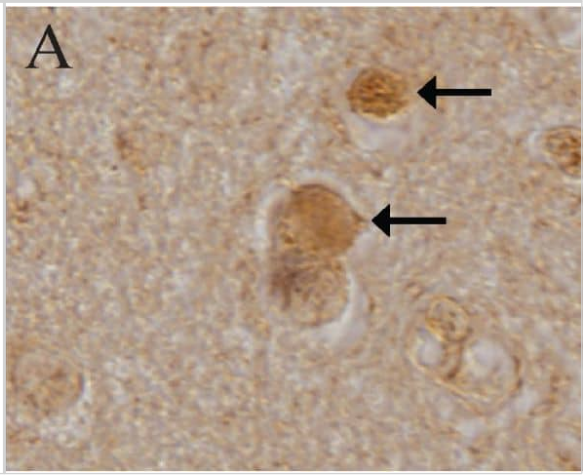
Immunohistochemistry-Paraffin: LRRK2 Antibody - BSA Free [NB300-268] - Immunocytochemistry of LRRK2 in DLB. Cortical LBs (arrows) in DLB were positive for LRRK2 using both Ab4 against LRRK22500–2527 (A), & Ab1 against LRRK2900–100 (B). Neuronal cytoplasm (C) was also strongly labelled throughout the cortex by Ab4 & with a consistently more granular pattern by Ab1 (inset in C) in cases of DLB. Control cases display similar, though less intense neuronal labeling (D). Biochemically purified cortical LBs were strongly positive for staining by Ab4 (E) as well as by anti-α-synuclein (F), while they were unstained when omitting primary antibody (G). Large vessels (H) were also intensely labelled by Ab4. Scale bars: A, B = 25 µm; C, D = 100 µm; E-G = 25 µm; H = 50 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/17137507), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

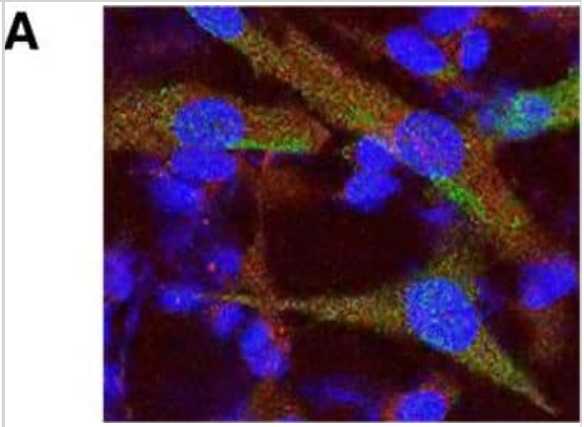
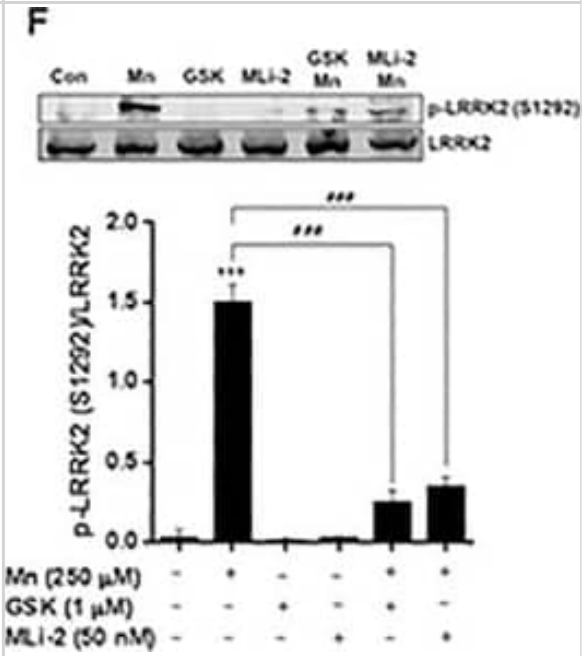


Immunohistochemistry-Paraffin: LRRK2 Antibody - BSA Free [NB300-268] - Immunocytochemistry of LRRK2 in DLB. Cortical LBs (arrows) in DLB were positive for LRRK2 using both Ab4 against LRRK22500–2527 (A), & Ab1 against LRRK2900–100 (B). Neuronal cytoplasm (C) was also strongly labelled throughout the cortex by Ab4 & with a consistently more granular pattern by Ab1 (inset in C) in cases of DLB. Control cases display similar, though less intense neuronal labeling (D). Biochemically purified cortical LBs were strongly positive for staining by Ab4 (E) as well as by anti-α-synuclein (F), while they were unstained when omitting primary antibody (G). Large vessels (H) were also intensely labelled by Ab4. Scale bars: A, B = 25 µm; C, D = 100 µm; E-G = 25 µm; H = 50 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/17137507), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

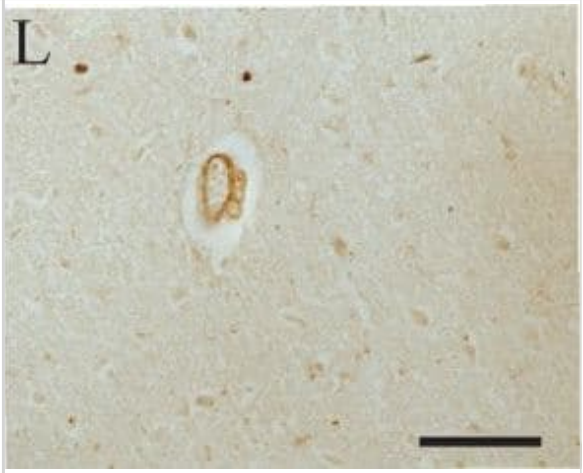


Immunohistochemistry-Paraffin: LRRK2 Antibody - BSA Free [NB300-268] - Immunocytochemistry of LRRK2 in DLB. Cortical LBs (arrows) in DLB were positive for LRRK2 using both Ab4 against LRRK22500–2527 (A), & Ab1 against LRRK2900–100 (B). Neuronal cytoplasm (C) was also strongly labelled throughout the cortex by Ab4 & with a consistently more granular pattern by Ab1 (inset in C) in cases of DLB. Control cases display similar, though less intense neuronal labeling (D). Biochemically purified cortical LBs were strongly positive for staining by Ab4 (E) as well as by anti-α-synuclein (F), while they were unstained when omitting primary antibody (G). Large vessels (H) were also intensely labelled by Ab4. Scale bars: A, B = 25 µm; C, D = 100 µm; E-G = 25 µm; H = 50 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/17137507), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: LRRK2 Antibody - BSA Free [NB300-268] - LRRK2 deletion attenuates Mn-induced pro-apoptotic protein levels.(A) After cells (LRRK2 WT & LRRK2 KO) were exposed to Mn (250 µM) for up to 24 h, total protein was extracted & followed by western blot analysis to determine protein levels of Bax & Daxx. β-actin was used as a loading control. (B) The expression levels of Bax & Daxx modulated by Mn were quantified relative to its control levels in LRRK2 WT & KO cells. **, p < 0.01; ***, p < 0.001 (one-way ANOVA followed by Tukey's post hoc test; n = 3). The data shown are representative of 3 independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30645642), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Immunocytochemistry/ Immunofluorescence: LRRK2 Antibody - BSA Free [NB300-268] - The interaction & phosphorylation of LRRK2 & LC1. (A) Cellular co-localisation of endogenous LRRK2 & MAP1B (LC1). SKNSH cells were labelled with LRRK2 (green) & LC1 (red) antibodies & signals were detected by immunofluorescence. Detailed figure in Additional file 1. (B) LRRK2 kinase assay. The LRRK2 wild-typed (WT) kinase interacted & phosphorylated the LC1 domain unlike the LRRK2 kinase-dead protein. (C) Western blotting of LRRK2 & LC1 proteins after a kinase assay. Both LRRK2 & LC1 proteins were probed for phospho-threonine & phospho-serine signals respectively. Image collected & cropped by CiteAb from the following publication (http://molecularbrain.biomedcentral.com/articles/10.1186/1756-6606-7-29), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: LRRK2 Antibody - BSA Free [NB300-268] - Mn increases LRRK2 kinase activity & expression in RAW 264.7 & HMC3 cells.(A) Two-hundred (200) µg of protein were collected from cell lysates, followed by western blot analysis to determine the presence of LRRK2 in LRRK2 WT, KO RAW 264.7 & HMC3 cells. β-actin was used as a loading control. (B,C) LRRK2 WT or HMC3 cells were treated with Mn (250 µM) for the designated times, followed by protein extraction & western blot analysis as described in the Methods section. Protein levels of LRRK2 & phosphorylated LRRK2 (S1292) in LRRK2 WT RAW 264.7 (B) & HMC3 (C) cells were quantified. (D,E) Effect of Mn on LRRK2 mRNA levels in LRRK2 WT RAW 264.7 (D) & HMC3 (E) cells were assessed as described in the Methods section. GAPDH was used as a loading control. (F) After pre-treatment with LRRK2 inhibitors GSK (1 µM) & MLi-2 (50 nM) for 90 min, RAW 264.7 cells were exposed to Mn (250 µM) for 20 min, followed by western blot analysis to detect phosphorylation of LRRK2 (S1292). ###, p < 0.001; *, p < 0.05; **, p < 0.01; ***, p < 0.001 compared to the control (one-way ANOVA followed by Tukey's post hoc test; n = 3). The data shown are representative of 3 independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30645642), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Immunohistochemistry-Paraffin: LRRK2 Antibody - BSA Free [NB300-268] - Immunocytochemistry of LRRK2 in PD. Four antibodies raised against sequences corresponding to various regions shown on the schematic diagram of LRRK2 (red bars) were used on brainstem sections of PD (A-H) & age-matched controls (I-L). Intense immunolabeling of brainstem LBs in cases of PD was seen with Ab1 against LRRK2900–100 (A) & Ab4 against LRRK22500–2527(D). Both rim (A & D) & core (inset in D) of LBs were recognized. In contrast, LBs were not labelled in any case using antibodies directed against LRRK21246–1265 (Ab2, B) or LRRK21838–2133 (Ab3, C), for which the antigenic sites are located within the folded domains. The cell bodies of both pigmented & no-pigmented neurons (arrows) as well as axons (arrowheads) contain LRRK2, seen only using antibodies against sites outside the folded domains (Ab1, E & Ab4, H). In contrast, Ab4 staining was much less intense in control tissue (L). The muscle layer of both large & small vessels was consistently found to contain high levels of LRRK2 in almost all PD cases (seen in E, G, & H) & strikingly the vessels are the only structure immunolabeled with Ab3 recognizing LRRK21838–2133 in all cases studied (G & K). Ab2 to LRRK21246–1265 did not recognize LBs, cell bodies or vessels in any case (B, F, & J). Scale bars: A-D & inset = 10 µm; E-L = 100 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/17137507), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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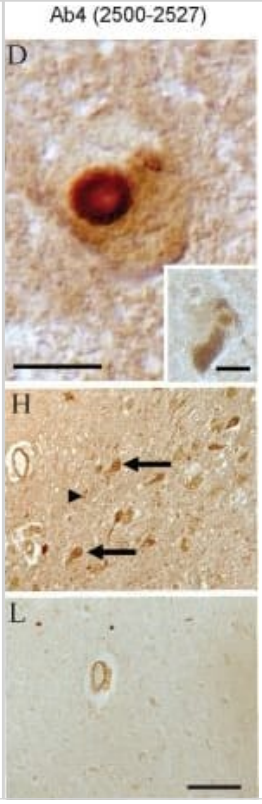
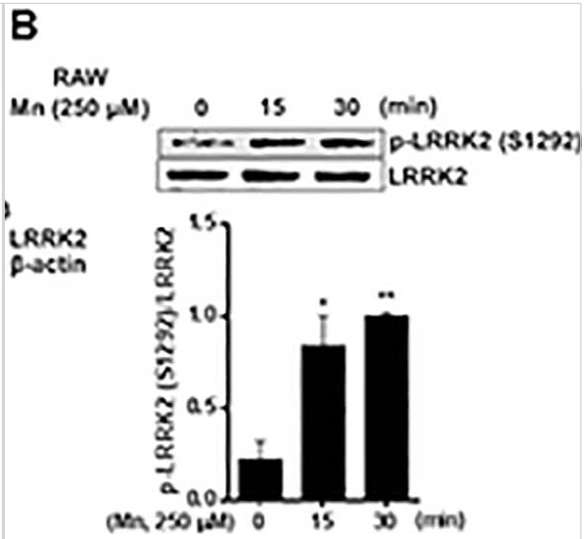
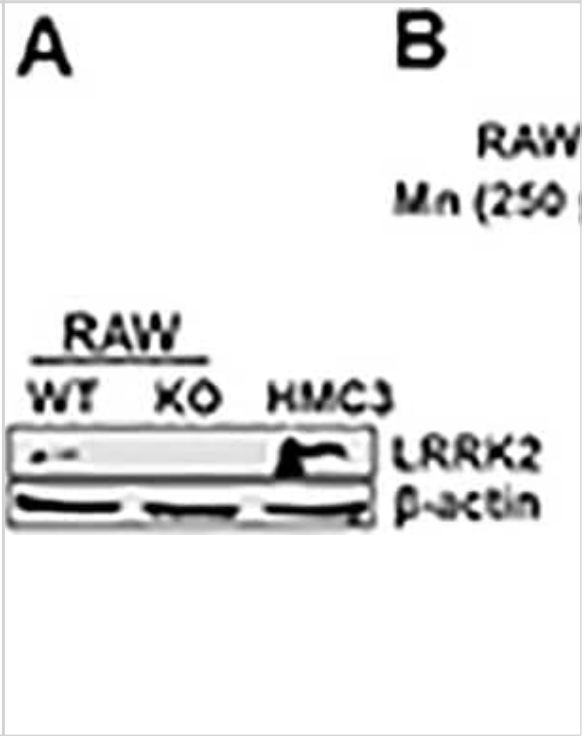
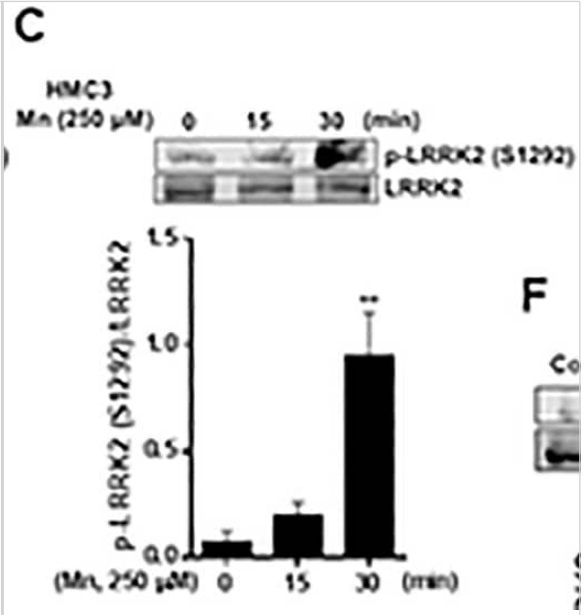
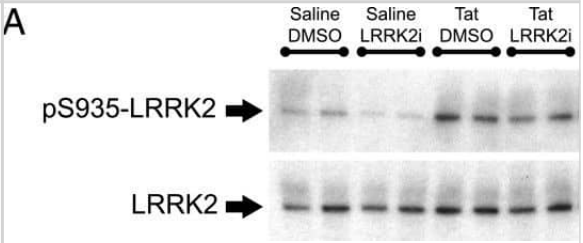


Western Blot: LRRK2 Antibody - BSA Free [NB300-268] - Mn increases LRRK2 kinase activity & expression in RAW 264.7 & HMC3 cells.(A) Two-hundred (200) µg of protein were collected from cell lysates, followed by western blot analysis to determine the presence of LRRK2 in LRRK2 WT, KO RAW 264.7 & HMC3 cells. β-actin was used as a loading control. (B,C) LRRK2 WT or HMC3 cells were treated with Mn (250 µM) for the designated times, followed by protein extraction & western blot analysis as described in the Methods section. Protein levels of LRRK2 & phosphorylated LRRK2 (S1292) in LRRK2 WT RAW 264.7 (B) & HMC3 (C) cells were quantified. (D,E) Effect of Mn on LRRK2 mRNA levels in LRRK2 WT RAW 264.7 (D) & HMC3 (E) cells were assessed as described in the Methods section. GAPDH was used as a loading control. (F) After pre-treatment with LRRK2 inhibitors GSK (1 µM) & MLi-2 (50 nM) for 90 min, RAW 264.7 cells were exposed to Mn (250 µM) for 20 min, followed by western blot analysis to detect phosphorylation of LRRK2 (S1292). ###, p < 0.001; *, p < 0.05; **, p < 0.01; ***, p < 0.001 compared to the control (one-way ANOVA followed by Tukey's post hoc test; n = 3). The data shown are representative of 3 independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30645642), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: LRRK2 Antibody - BSA Free [NB300-268] - Mn increases LRRK2 kinase activity & expression in RAW 264.7 & HMC3 cells.(A) Two-hundred (200) µg of protein were collected from cell lysates, followed by western blot analysis to determine the presence of LRRK2 in LRRK2 WT, KO RAW 264.7 & HMC3 cells. β-actin was used as a loading control. (B,C) LRRK2 WT or HMC3 cells were treated with Mn (250 µM) for the designated times, followed by protein extraction & western blot analysis as described in the Methods section. Protein levels of LRRK2 & phosphorylated LRRK2 (S1292) in LRRK2 WT RAW 264.7 (B) & HMC3 (C) cells were quantified. (D,E) Effect of Mn on LRRK2 mRNA levels in LRRK2 WT RAW 264.7 (D) & HMC3 (E) cells were assessed as described in the Methods section. GAPDH was used as a loading control. (F) After pre-treatment with LRRK2 inhibitors GSK (1 µM) & MLi-2 (50 nM) for 90 min, RAW 264.7 cells were exposed to Mn (250 µM) for 20 min, followed by western blot analysis to detect phosphorylation of LRRK2 (S1292). ###, p < 0.001; *, p < 0.05; **, p < 0.01; ***, p < 0.001 compared to the control (one-way ANOVA followed by Tukey's post hoc test; n = 3). The data shown are representative of 3 independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30645642), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Western Blot: LRRK2 Antibody - BSA Free [NB300-268] - Mn increases LRRK2 kinase activity & expression in RAW 264.7 & HMC3 cells.(A) Two-hundred (200) µg of protein were collected from cell lysates, followed by western blot analysis to determine the presence of LRRK2 in LRRK2 WT, KO RAW 264.7 & HMC3 cells. β-actin was used as a loading control. (B,C) LRRK2 WT or HMC3 cells were treated with Mn (250 µM) for the designated times, followed by protein extraction & western blot analysis as described in the Methods section. Protein levels of LRRK2 & phosphorylated LRRK2 (S1292) in LRRK2 WT RAW 264.7 (B) & HMC3 (C) cells were quantified. (D,E) Effect of Mn on LRRK2 mRNA levels in LRRK2 WT RAW 264.7 (D) & HMC3 (E) cells were assessed as described in the Methods section. GAPDH was used as a loading control. (F) After pre-treatment with LRRK2 inhibitors GSK (1 µM) & MLi-2 (50 nM) for 90 min, RAW 264.7 cells were exposed to Mn (250 µM) for 20 min, followed by western blot analysis to detect phosphorylation of LRRK2 (S1292). ###, p < 0.001; *, p < 0.05; **, p < 0.01; ***, p < 0.001 compared to the control (one-way ANOVA followed by Tukey's post hoc test; n = 3). The data shown are representative of 3 independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30645642), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: LRRK2 Antibody - BSA Free [NB300-268] - LRRK2 kinase inhibition blocks Tat-induced S935 phosphorylation & inflammatory cytokine expression in BV-2 cells. (A) Western blot depicting pS935-LRRK2 & total LRRK2 protein levels 12 hours after saline or Tat-treatment ± LRRK2 kinase inhibition (LRRK2i). (B) Tat treatment significantly increased pS935-LRRK2, which was attenuated by LRRK2i. (C) We measured TNF-α, IL-6, MCP-1 & IL-10 mRNA levels by qRT-PCR 12 hours post saline or Tat treatment ± LRRK2i. LRRK2i attenuated Tat-induced TNF-α, IL-6, MCP-1 & IL-10 expression for all groups (*P <0.05, ***P <0.001, one way ANOVA, Newman-Keuls post-test). ANOVA, analysis of variance; LRRK2, leucine-rich repeat kinase2; pS935, phosphorylation of serine 935; Tat, trans activator of transcription. Image collected & cropped by CiteAb from the following publication (http://jneuroinflammation.biomedcentral.com/articles/10.1186/1742-2094-9-261), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

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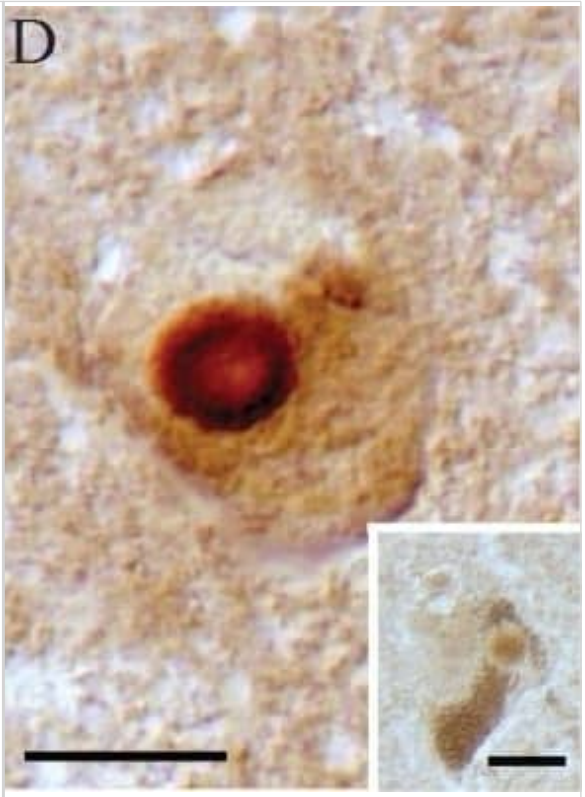
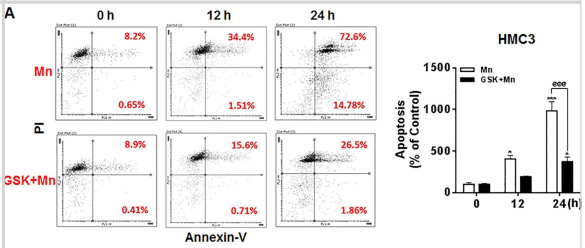


Flow Cytometry: LRRK2 Antibody - BSA Free [NB300-268] - Inhibition of LRRK2 kinase activity attenuates Mn-induced apoptosis.(A) After pre-treatment with GSK (1 µM) for 90 min, cells (HMC3) were exposed to Mn (250 µM) for the designated time periods, followed by annexin V & PI staining & flow cytometry analysis to determine apoptosis. Early & late apoptotic cells (Q2 & Q3) were analyzed. (B,C) After pre-treatment with GSK (1 µM) for 90 min, cells (LRRK2 WT RAW 264.7 & HMC3) were exposed to Mn for designated time periods, followed by the MTT assay to determine cell viability, as described in the Methods section, (@@@, $p < 0.001$; *, $p < 0.05$; ***, $p < 0.001$ compared to the control (one-way ANOVA followed by Tukey's post hoc test; n = 3, apoptosis assay; n = 6, MTT assay). The data shown are representative of 3 independent experiments. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30645642), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Wang H, Li J, Tao L et al. MiR-205 Regulates LRRK2 Expression in Dopamine Neurons in Parkinson's Disease through Methylation Modification Iranian Journal of Public Health 2022-07-17 [PMID: 36248294]

Pajarillo E, Kim SH, Digman A et al. The role of microglial LRRK2 kinase in manganese-induced inflammatory neurotoxicity via NLRP3 inflammasome and RAB10-mediated autophagy dysfunction The Journal of biological chemistry 2023-06-01 [PMID: 37269951] (Mouse)

Pajarillo E, Kim SH, Digman A et al. The role of microglial LRRK2 in manganese-induced inflammatory neurotoxicity via NLRP3 inflammasome and RAB10-mediated autophagy dysfunction bioRxiv : the preprint server for biology 2023-04-05 [PMID: 37066140] (IHC, Mouse)

Wallings RL, Hughes LP, Staley HA Et al. WHOPPA Enables Parallel Assessment of Leucine-Rich Repeat Kinase 2 and Glucocerebrosidase Enzymatic Activity in Parkinson's Disease Monocytes Front Cell Neurosci 2022-06-27 [PMID: 35755775] (FLOW, Human)

Details:
Citation using the Alexa Fluor 700 version of this antibody.

Vitte, J, Traver, S Et al. Leucine-rich repeat kinase 2 is associated with the endoplasmic reticulum in dopaminergic neurons and accumulates in the core of Lewy bodies in Parkinson disease. J Neuropathol Exp Neurol 2010-09-01 [PMID: 20720502] (ICC/IF, Human)

Bharat V, Hsieh CH, Wang X Mitochondrial Defects in Fibroblasts of Pathogenic MAPT Patients Frontiers in cell and developmental biology 2021-11-03 [PMID: 34805172] (WB, Human)

Chen Z C, Zhang W et al. Phosphorylation of amyloid precursor protein by mutant LRRK2 promotes AICD activity and neurotoxicity in Parkinson's disease. Sci Signal 2017-07-18 [PMID: 28720718] (IP, Mouse)

Chen S, Luo Z, Ward C et al. Generation of two LRRK2 homozygous knockout human induced pluripotent stem cell lines using CRISPR/Cas9 Stem Cell Res 2020-04-21 [PMID: 32339904] (WB, Human)

Lim J, Bang Y, Choi JH et al. LRRK2 G2019S Induces Anxiety/Depression-like Behavior before the Onset of Motor Dysfunction with 5-HT1A Receptor Upregulation in Mice J Neurosci. 2018-02-13 [PMID: 29305532] (IHC-P, Mouse)

Hsieh CH, Li L, Vanhauwaert R et al. Miro1 Marks Parkinson\'s Disease Subset and Miro1 Reducer Rescues Neuron Loss in Parkinson\'s Models Cell Metab. 2019-09-23 [PMID: 31564441]

Kim J, Pajarillo E, Rizor A et al. LRRK2 kinase plays a critical role in manganese-induced inflammation and apoptosis in microglia. PLoS ONE 2019-01-15 [PMID: 30645642] (WB, Mouse)

Bonello F, Hassoun SM, Mouton-Liger F et al. LRRK2 impairs PINK1/Parkin-dependent mitophagy via its kinase activity: pathologic insights into Parkinson's disease. Hum. Mol. Genet. 2019-01-09 [PMID: 30629163] (ICC/IF, Human)

More publications at http://www.novusbio.com/NB300-268

Procedures

Immunoprecipitation protocol for LRRK2 Antibody (NB300-268)

Protocol for immunoprecipitation of LRRK2 followed by LRRK2 autophosphorylation kinase assay

Cell lysis
3X15 cm plates of SH-SY5Y cells were grown to 80% confluency. The plates were washed twice with PBS and placed on ice. Remaining PBS was aspirated off after tilting plate to remove all PBS. 1.5 ml of cold lysis buffer (buffer A) was added to each plate. The plates were allowed to incubate on ice 5 min until the cells detached. The lysis buffer and cells for each plate were then vigorously passed 6X through a 30.5 guage needle. Lysis buffer and cells were transferred to 3X1.5 ml eppendorf tubes and spun 5 min. at 5,000 rpm in a 4 degree eppendorf microfuge. Lysates were removed from pelleted debris and transferred to new 1.5 ml eppendorf tubes and recentrifuged 10 min. at 13,000 rpm. Lysate was transferred to three new tubes and 1/2 lysate volume of buffer A (-) NaCl was added to each tube.
Preclear
10 ug of rabbit IgG were added to the lysate for each tube and the lysate was vortexed followed by rotating at 4 degrees for 2 hours. 20 ul of protein A sepharose beads (Amersham cat#: 17-0469-01) were added. The tubes were vortexed and then rotated for 1.5 hours at 4 degrees. Lysates were separated from protein A beads beads by low (200 rpm) spin for 2 min and transferred to new eppendorf tubes. A repeat of the protein A sepharose incubation was carried out to remove residual rabbit IgG followed by removal of the protein A beads.

Immunoprecipitation with LRRK2 Ab

To two of the tubes containing precleared lysate were added 7 ul of LRRK2 Ab (7ug). To the remaining tube was added 7ug of rabbit IgG. The tubes were vortexed and allowed to rotate overnight at 4 degrees. The following morning 30 ul of protein A sepharose was added to each tube, the tubes were vortexed and rotated at 4 degrees for 2 hours. The protein A beads were then isolated by brief, low speed centrifugation and were washed 3X in 500ul buffer A (-) NaCl. This was followed by two washes in kinase buffer (buffer B). Protein A beads were resuspended in 1 volume (30 ul) of buffer B for a total of 60ul of immunoprecipitate.
Autophosphorylation kinase reaction, gel electrophoresis and phosphoimaging
On ice, 40 ul of immunoprecipitate from each tube was transferred to a .5ml kinase reaction tube. Each of the three reactions was supplemented with a 5 ul mixture that gave a final reaction concentration of 15 uM cold ATP and 5uCi ATP. The reaction mixtures were vortexed and transferred to a rotator in a 30 degree incubator. The autophosphorylation incubation was allowed to go for 30 minutes and the reaction tubes were taken off the rotator and vortexed every five minutes. The reactions were then halted by addition of 11ul of 5X SDS gel running sample buffer to each of the three samples. 40ul of each sample was then run on a 7% acrylamide-acetate mini-gel. Once the 200Kd molecular weight marker band had run half way down the gel, the gel was stopped dried and exposed blanked to a phosphoimaging cassette (Molecular Dynamics). Following 24 hour exposure, the cassette was assessed for radioactivity on a Storm analyzer.

Buffers

Buffer A (make 10ml both - and + NaCl solutions) = lysis buffer
50mM Tris pH 7.4
150mM NaCl
0.2% NP40
Protease inhibitor cocktail (stock = 100X, Sigma)
0.5mM vanadate
15mM EDTA
adjust to 10 ml with H2O
Buffer B = kinase buffer
10mM hepes
10mM MgCl2
50mM NaCl
protease inhibitor
vanadate
(1mM NaN3 if storing overnight or longer)

Immunohistochemistry-Paraffin protocol for LRRK2 Antibody (NB300-268)
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.

Immunocytochemistry/ Immunofluorescence Protocol for LRRK2 Antibody (NB300-268)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

Flow (Intracellular) Protocol for LRRK2 Antibody (NB300-268)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.

a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.

4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabilization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.

a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.

b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).

3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

Western Blot Protocol for LRRK2 Antibody (NB300-268)
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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