# Product Datasheet

## LRRK2 Antibody
## NB300-268SS

Unit Size: 0.025 ml

Store at 4C. Do not freeze.



**Reviews: 1 Publications: 21**

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# NB300-268SS

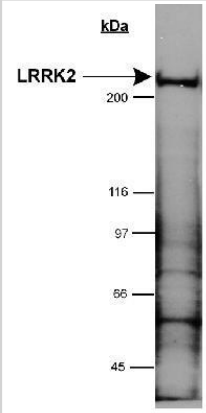
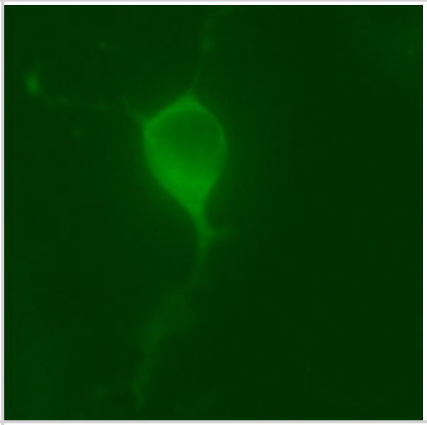
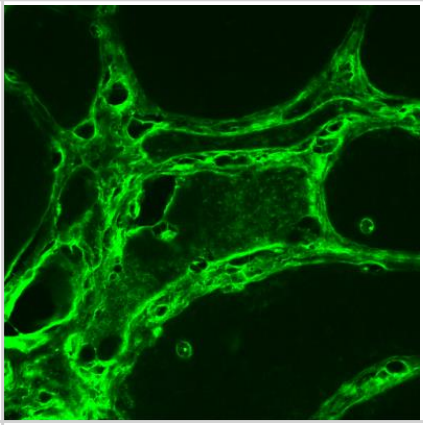
LRRK2 Antibody

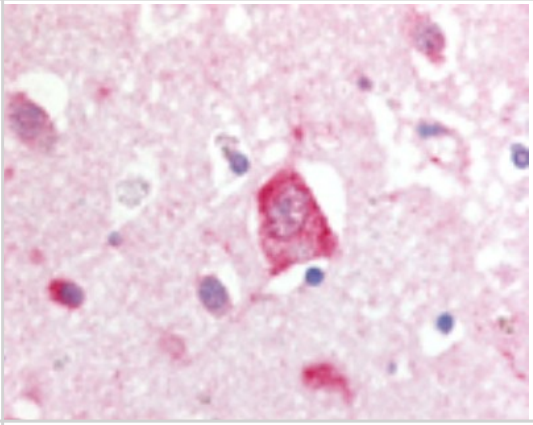
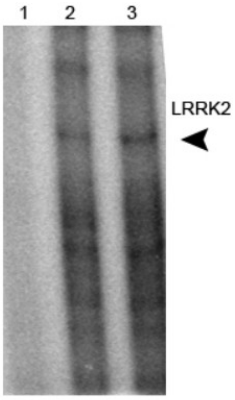
| Product Information | |
|---|---|
| **Unit Size** | 0.025 ml |
| **Concentration** | 1 mg/ml |
| **Storage** | Store at 4C. Do not freeze. |
| **Clonality** | Polyclonal |
| **Preservative** | 0.1% Sodium Azide |
| **Purity** | Immunogen affinity purified |
| **Buffer** | Tris-citrate/phosphate, pH 7-8 |
| **Target Molecular Weight** | 286 kDa |

| Product Description | |
|---|---|
| **Host** | Rabbit |
| **Gene ID** | 120892 |
| **Gene Symbol** | LRRK2 |
| **Species** | Human, Mouse, Bovine, C. elegans |
| **Species Reactivity** | Human, mouse, bovine, C. elegans, Drosophila and moth. Immunogen sequence has 87% homology to rat. |
| **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, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation |
| **Recommended Dilutions** | Immunocytochemistry/Immunofluorescence 1:1000-1:2000, Immunohistochemistry 1:500-1:1000, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin 1:500-1:1000, Immunoprecipitation 1:10-1:500, Western Blot 1:2000-1:5000 |
| **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. |

## Images

| | |
|---|---|
| Western Blot: LRRK2 Antibody [NB300-268] - Detection of LRRK2 in HeLa whole cell lysate (RIPA) using NB300-268. 1:5000 dilution, 1 minute ECL detection. |   |
| Immunocytochemistry/Immunofluorescence: LRRK2 Antibody [NB300-268] - Mouse CAD cells transfected with Human wild-type LRRK-2 (1:2,000). |  |
| Immunohistochemistry-Frozen: LRRK2 Antibody [NB300-268] - Staining as described in PMID 24312256. Image from confirmed customer review. |  |
| Western Blot: LRRK2 Antibody [NB300-268] - Detection of LRRK2 in 50 ug of crude bovine brain membrane. |   |

| | |
|---|---|
| Immunohistochemistry: LRRK2 Antibody [NB300-268] - Brain, Putamen, Neurons and Glia 60x. |  |
| Immunoprecipitation: LRRK2 Antibody [NB300-268] - Immunoprecipitation of LRRK2 followed by an autophosphorylation assay using NB300-268. Lane 1 Rabbit IgG, Lanes 2 and 3 NB300-268. |   |

## Publications

Chan SL, Chua LL, Angeles DC et al. MAP1B rescues LRRK2 mutant-mediated cytotoxicity. Mol Brain 2014 Apr 25 [PMID: 24754922] (ICC/IF, WB, Human)

Angeles DC, Ho P, Chua LL et al. Thiol peroxidases ameliorate LRRK2 mutant-induced mitochondrial and dopaminergic neuronal degeneration in Drosophila. Hum. Mol. Genet. 2014 Feb 27 [PMID: 24459295] (WB, Human)

Sekigawa A, Fujita M, Sekiyama K et al. Distinct mechanisms of axonal globule formation in mice expressing human wild type alpha-synuclein or dementia with Lewy bodies-linked P123H beta-synuclein. Mol Brain. 2012 Sep 26 [PMID: 23013868] (IHC-Fr , Mouse)

Greggio E, Lewis PA, van der Brug MP et al. Mutations in LRRK2/dardarin associated with Parkinson disease are more toxic than equivalent mutations in the homologous kinase LRRK1. J Neurochem. 2007 Jul [PMID: 17394548] (WB, Human)

Kamikawaji S, Ito G, Sano T, Iwatsubo T. Differential Effects of Familial Parkinson Mutations in LRRK2 Revealed by a Systematic Analysis of Autophosphorylation. Biochemistry. 2013 Aug 23 [PMID: 23924436] (WB, Human)

Kamikawaji S, Ito G, Iwatsubo T. Identification of the autophosphorylation sites of LRRK2. Biochemistry 2009 Nov 24 [PMID: 19824698] (IP, WB, Human, Moth)

Qing H, Zhang Y, Deng Y et al. Lrrk2 interaction with alpha-synuclein in diffuse Lewy body disease. Biochem Biophys Res Commun 2009 Dec 25 [PMID: 19878656] (IHC, ICC/IF, Human)

Marker DF, Puccini JM, Mockus TE et al. LRRK2 kinase inhibition prevents pathological microglial phagocytosis in response to HIV-1 Tat protein J Neuroinflammation 2012 Nov 29 [PMID: 23190742] (ICC/IF, Mouse)

Yao C, El Khoury R, Wang W et al. LRRK2-mediated neurodegeneration and dysfunction of dopaminergic neurons in a Caenorhabditis elegans model of Parkinson's disease Neurobiol Dis 2010 Oct [PMID: 20382224] (IHC, ICC/IF, WB, C. elegans)

Pandey N, Fahey MT, Jong YJ, O'Malley KL. Sequences Located within the N-Terminus of the PD-Linked LRRK2 Lead to Increased Aggregation and Attenuation of 6-Hydroxydopamine-Induced Cell Death PLoS One 2012 [PMID: 23028814] (WB, Human)

Hermann A et al. Reduced LRRK2-positive neurons in the striatum of Parkinson’s disease patients hypothesize a retrograde disease mechanism? . Basal Ganglia http://dx.doi.org/10.1016/j.baga.2012.04.030. 2012 (IHC, ICC/IF, Human)

Liu Z, Lee J, Krummey S et al. The kinase LRRK2 is a regulator of the transcription factor NFAT that modulates the severity of inflammatory bowel disease. Nat Immunol;12(11):1063-1070. 2011 [PMID: 21983832]

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

## Procedures

**Protocol specific for LRRK2 Antibody (NB300-268)**
Immunofluorescence Protocol:

1. Mouse CAD cells were transfected with Human wild-type LRRK-2.
2. 48hr following transfection, the cells were fixed for 10 min with ice-cold MeOH.
3. The cells were washed 3X with PBS, blocked with 10% normal donkey serum (NDS) in PBS containing 0.25% Triton for 1 hr at room temperature.
4. The cells were incubated overnight at 4C with rabbit anti-LRRK2 (Cat. # NB 300-268) diluted 1/2000 in 1% NDS in PBS containing 0.25% Triton.
5. The following day, the cells were washed 5X with PBS and incubated with FITC conjugated donkey anti-rabbit (1/100) diluted in 1% NDS in PBS containing 0.25% Triton for 1 hr.
6. The cells were washed 5X with PBS and mounted with Vectashield, and visualized with a 40X oil-immersion objective.

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)

IHC-FFPE sections
I. Deparaffinization:
A. Treat slides with Xylene: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.
B. Treat slides with 100% Reagent Alcohol: 3 changes for 5 minutes each. Drain slides for 10 seconds between changes.

II. Quench Endogenous Peroxidase:
A. Place slides in peroxidase quenching solution: 15-30 minutes.
To Prepare 200 ml of Quenching Solution:
Add 3 ml of 30% Hydrogen Peroxide to 200 ml of Methanol.
Use within 4 hours of preparation
B. Place slides in distilled water: 2 changes for 2 minutes each.

III. Retrieve Epitopes:
A. Preheat Citrate Buffer. Place 200 ml of Citrate Buffer Working Solution into container, cover and place into steamer. Heat to 90-96 degrees Celcius.
B. Place rack of slides into hot Citrate Buffer for 20 minutes. Cover.
C. Carefully remove container with slides from steamer and cool on bench, uncovered, for 20 minutes.
D. Slowly add distilled water to further cool for 5 minutes.
E. Rinse slides with distilled water. 2 changes for 2 minutes each.

IV. Immunostaining Procedure:
A. Remove each slide from rack and circle tissue section with a hydrophobic barrier pen (e.g. Liquid Blocker-Super Pap Pen).
B. Flood slide with Wash Solution. Do not allow tissue sections to dry for the rest of the procedure.
C. Drain wash solution and apply 4 drops of Blocking Reagent to each slide and incubate for 15 minutes.
D. Drain Blocking Reagent (do not wash off the Blocking Reagent), apply 200 ul of Primary Antibody solution to each slide, and incubate for 1 hour.
E. Wash slides with Wash Solution: 3 changes for 5 minutes each.
F. Drain wash solution, apply 4 drops of Secondary antibody to each slide and incubate for 1 hour.
G. Wash slides with Wash Solution: 3 changes for 5 minutes each.
H. Drain wash solution, apply 4 drops of DAB Substrate to each slide and develop for 5-10 minutes. Check development with microscope.
I. Wash slides with Wash Solution: 3 changes for 5 minutes each.
J. Drain wash solution, apply 4 drops of Hematoxylin to each slide and stain for 1-3 minutes. Increase time if darker counterstaining is desired.
K. Wash slides with Wash Solution: 2-3 changes for 2 minutes each.
L. Drain wash solution and apply 4 drops of Bluing Solution to each slide for 1-2 minutes.
M. Rinse slides in distilled water.
N. Soak slides in 70% reagent alcohol: 3 minutes with intermittent agitation.
O. Soak slides in 95% reagent alcohol: 2 changes for 3 minutes each with intermittent agitation.
P. Soak slides in 100% reagent alcohol: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds between each change.
Q. Soak slides in Xylene: 3 changes for 3 minutes each with intermittent agitation. Drain slides for 10 seconds

between each change.
R. Apply 2-3 drops of non-aqueous mounting media to each slide and mount coverslip.
S. Lay slides on a flat surface to dry prior to viewing under microscope.

NOTES:
-Use treated slides (e.g. HistoBond) to assure adherence of FFPE sections to slide.
-Prior to deparaffinization, heat slides overnight in a 60 degrees Celcius oven.
-All steps in which Xylene is used should be performed in a fume hood.
-For Epitope Retrieval, a microwave or pressure cooker may be substituted for the steamer method. Adjust times as necessary depending on conditions.
-For the initial IHC run with a new primary antibody, test tissues with and without Epitope Retrieval. In some instances, Epitope Retrieval may not be necessary.
-200 ul is the recommended maximum volume to apply to a slide for full coverage. Using more than 200 ul may allow solutions to wick off the slide and create drying artifacts. For small tissue sections less than 200 ul may be used.
-5 minutes of development with DAB Substrate should be sufficient. Do not develop for more than 10 minutes. If 5 minutes of development causes background staining, further dilution of the primary antibody may be necessary.
-Hematoxylin should produce a light nuclear counterstain so as not to obscure the DAB staining. Counterstain for 1-1 1/2 minutes for nuclear antigens. Counterstain for 2-3 minutes for cytoplasmic and membranous antigens. If darker counterstaining is desired increase time (up to 10 minutes).

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**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 guarantee, please visit www.novusbio.com/guarantee.**