Product Datasheet

LRRK2 Antibody
NB300-268

Unit Size: 0.1 ml
Store at 4C. Do not freeze.

Reviews: 1  Publications: 38

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# NB300-268
## LRRK2 Antibody

### Product Information

<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit Size</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Concentration</td>
<td>1 mg/ml</td>
</tr>
<tr>
<td>Storage</td>
<td>Store at 4°C. Do not freeze.</td>
</tr>
<tr>
<td>Clonality</td>
<td>Polyclonal</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.1% Sodium Azide</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG</td>
</tr>
<tr>
<td>Purity</td>
<td>Immunogen affinity purified</td>
</tr>
<tr>
<td>Buffer</td>
<td>Tris-Citrate/Phosphate (pH 7.0 - 8.0)</td>
</tr>
<tr>
<td>Target Molecular Weight</td>
<td>286 kDa</td>
</tr>
</tbody>
</table>

### Product Description

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

### Product Application Details

<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applications</td>
<td>Western Blot, Flow Cytometry, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation</td>
</tr>
<tr>
<td>Application Notes</td>
<td>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.</td>
</tr>
</tbody>
</table>
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.


Immunocytochemistry/Immunofluorescence: LRRK2 Antibody [NB300-268] - Mouse CAD cells transfected with Human wild-type LRRK-2 (1:2,000).

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.

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

Publications


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. Sep 23 2019 12:00AM [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 Jan 15 2019 12:00AM [PMID: 30645642] (WB, Mouse)


Nucifora FC, Nucifora LG, Ng CH et al. Ubiquitination via K27 and K29 chains signals aggregation and neuronal protection of LRRK2 by WSB1. Nat Commun Jun 9 2016 12:00AM [PMID: 27273569]


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 gauge 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)

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 Celsius.
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).
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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