Product Datasheet

Park7/DJ-1 Antibody - BSA Free
NB300-270SS

Unit Size: 0.025 ml

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

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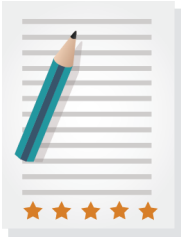
Updated 4/13/2025 v.20.1



NB300-270SS

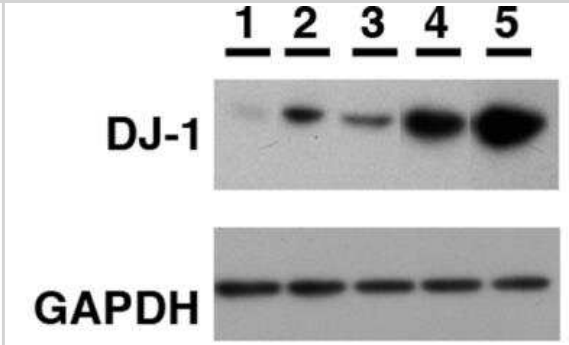
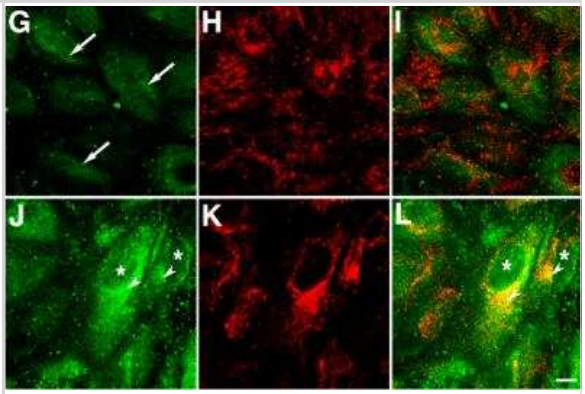
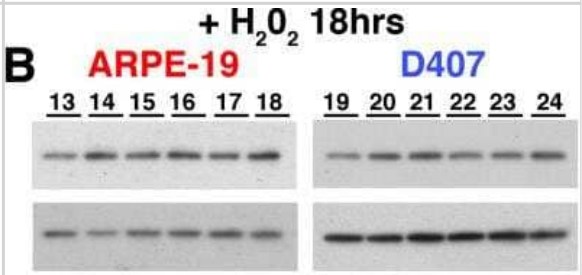
Park7/DJ-1 Antibody - BSA Free

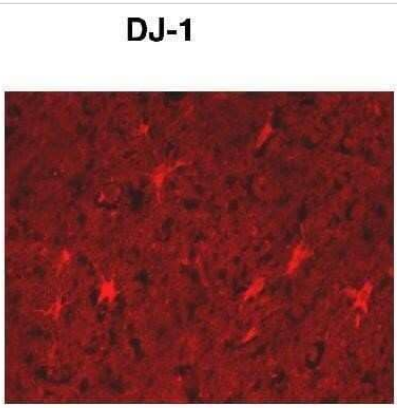
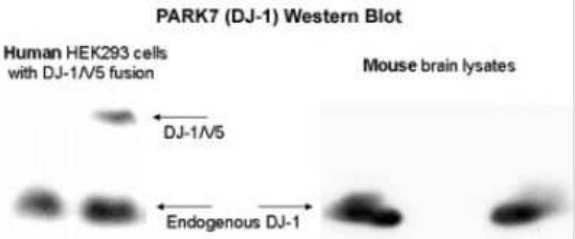
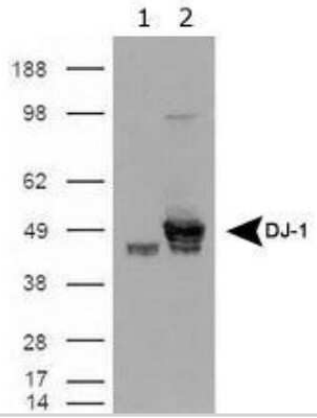
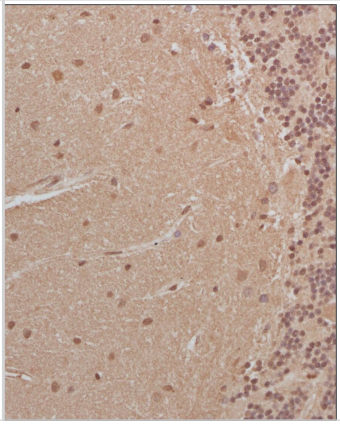
Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	25 kDa

Product Description	
Host	Rabbit
Gene ID	11315
Gene Symbol	PARK7
Species	Human, Mouse, Rat, Chicken
Reactivity Notes	Predicted to react with bovine, Zebra fish, and Golden Syrian hamster based on 100% sequence homology. Chicken reactivity reported in scientific literature (PMID: 24064392). Rat reactivity reported in scientific literature (PMID: 26419955)
Immunogen	A synthetic peptide corresponding to the C-terminus (within residues 150-189) of human Park7(DJ-1). [UniProt# Q99497]

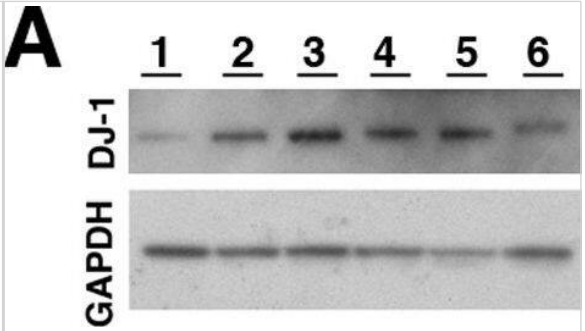
Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, In vitro assay, Immunoprecipitation
Recommended Dilutions	Western Blot 1:2000, Simple Western 1:4000, Flow Cytometry reported in scientific literature (PMID 21937704), Immunohistochemistry, Immunocytochemistry/ Immunofluorescence 1:500, Immunoprecipitation 1:200, Immunohistochemistry-Paraffin, In vitro assay reported in scientific literature (PMID 21645620)
Application Notes	In Western Blot, a band can be seen at approx. 25 kDa. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in Human Brain lysate 0.05 mg/mL, separated by Size, antibody dilution of 1:4000, apparent MW was 26 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

Images

Simple Western: Park7/DJ-1 Antibody [NB300-270] - Simple Western lane view shows a specific band for Park7 (DJ-1) in 0.05 mg/ml of Human Brain lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Western Blot: Park7/DJ-1 Antibody [NB300-270] - Lysates of the human RPE cell lines ARPE-19 (lane 1) and D407 (lane 2), the mouse RPE cell line B6-RPE07 (lane 3), mouse primary RPE (lane 4) and mouse brain lysates (lane 5) were harvested and analyzed by immunoblot assay with DJ-1 antibody. The DJ-1 signal varied in intensity in each RPE cell culture. Each lane contained 20 ug of protein. Protein loads were confirmed in replicate blots probed with GAPDH. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0067983), licensed under a CC-BY license.	
Immunocytochemistry/Immunofluorescence: Park7/DJ-1 Antibody [NB300-270] - Representative confocal micrographs of ARPE-19 monolayers plated on Transwells(R) and labeled with antibodies to DJ-1 (G,J) and COX IV (H,K). Under baseline conditions, there is very little colocalization between DJ-1 and COX IV, as observed in overlaid images (I); DJ-1 is mostly distributed through the cytoplasm (arrows). Upon oxidative stress induced by incubation with 400 uM H2O2 for 1 hr, DJ-1 staining is increased (arrowheads) in ARPE-19 (J) cultures. In cultures treated with H2O2 some DJ-1 re-distributed to mitochondria (arrowheads) and displayed significant colocalization with COX IV in overlaid images (L). Scale bar = 10 um. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0067983), licensed under a CC-BY license.	
Western Blot: Park7/DJ-1 Antibody [NB300-270] - ARPE-19 and D407 monolayers were treated with increasing concentrations (0 to 800 uM) of H2O2 for 18 hrs (B), harvested, and analyzed by immunoblot assay with DJ-1 antibody (upper panel). Each lane contained 20 ug of protein. Protein loadings were confirmed in replicate blots probed with GAPDH (lower panel). A representative Western is shown. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0067983), licensed under a CC-BY license	

Immunohistochemistry-Paraffin: Park7/DJ-1 Antibody [NB300-270] - Park7(DJ-1) detected in paraffin embedded human cortex.	
Western Blot: Park7/DJ-1 Antibody [NB300-270] - Human HEK293 cells and mouse brain lysates.	
Western Blot: Park7/DJ-1 Antibody [NB300-270] - Cells were transfected with the pCMV6-ENTRY control (lane 1) or pCMV6-ENTRY PARK7 cDNA (lane 2) for 48 hrs and lysed. Equivalent amounts of cell lysates (5 ug per lane) were separated by SDS-PAGE and immunoblotted with anti-Park7(DJ-1).	
Analysis of a FFPE tissue section of mouse brain using 1:200 dilution of Park7/DJ-1 antibody. The staining was developed using HRP labeled anti-rabbit secondary antibody and DAB reagent, and nuclei of cells were counter-stained with hematoxylin.	

Western Blot: Park7/DJ-1 Antibody [NB300-270] - Presence of oxDJ-1 in RPE cells subjected to oxidative stress.ARPE-19 monolayers were treated with increasing concentrations (0 to 800 µM) of H2O2 for 1 hr (A) & 18 hs (B), harvested, & analyzed by immunoblot assay with oxDJ-1 antibody (upper panel). Protein loadings were confirmed in replicate blots probed with GAPDH (lower panel). Each lane contained 20 µg of protein. A dose response is observed when cells are exposed to increasing concentrations of H2O2 for 1 h (A, lanes 1 to 6) & 18 hrs (B, lanes 7 to 12). Confocal immunofluorescence staining of baseline ARPE-19 cultures (C) fixed before extraction with Triton X-100 & labeling with oxDJ-1 antibodies revealed absence of oxDJ-1. However, oxDJ-1 is observed in the cytoplasm (arrows) & perinuclear area (arrowheads) of RPE cells exposure to 400 µM H2O2 for 1 h (D) & 18 hrs (E). Cell nuclei were labeled with TO-PRO-3. Scale bar=20 µm. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/23844142), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



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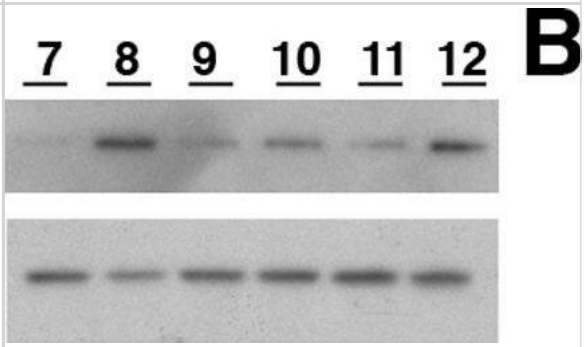

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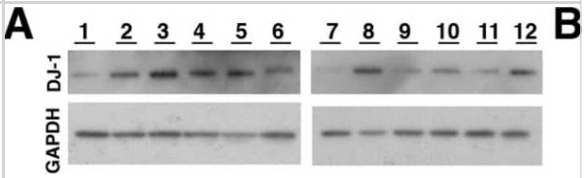

Publications

Bhattacharyya S, Sturgis J, Maminishkis A et al. Oxidation of DJ-1 Cysteines in Retinal Pigment Epithelium Function International Journal of Molecular Sciences 2022-09-01 [PMID: 36077335]

Riou C, Brionne A, Cordeiro L et al. Avian uterine fluid proteome: Exosomes and biological processes potentially involved in sperm survival Molecular Reproduction and Development 2020-04-01 [PMID: 32350983] (Immunohistochemistry, Western Blot, Electron Microscopy)

Gharbi N, R□ise D, F□rre JE et al. Reintroduction of DJ-1 in M□ller Cells Inhibits Retinal Degeneration in the DJ-1 Deficient Retina Antioxidants (Basel) 2021-11-23 [PMID: 34942966] (Immunohistochemistry-Frozen)

Wolk AM The Role of the Retinal Pigment Epithelium in Sorsby Fundus Dystrophy Thesis 2021-01-01 (WB, Mouse)

Sikorski K, Mehta A et al. A high-throughput pipeline for validation of antibodies. Nat Methods 2018-01-11 [PMID: 30377371] (Human)

Details:
Antibody validation based on denaturing gel electrophoresis of biotinylated cell lysates (PAGE) followed by mass spectrometry (MS) and antibody array analysis (MAP).

Wolk A, Upadhyay M, Ali M et al. The retinal pigment epithelium in Sorsby Fundus Dystrophy shows increased sensitivity to oxidative stress-induced degeneration Redox Biol 2020-08-10 [PMID: 32828705] (WB, Mouse)

Upadhyay M, Milliner C, Bell B, Bonilha V Oxidative stress in the retina and retinal pigment epithelium (RPE): Role of aging, and DJ-1 Redox Biology 2020-07-01 [PMID: 32826201] (WB, Mouse)

Edson AJ, Hushagen HA, Froyset AK, et al. Dysregulation in the Brain Protein Profile of Zebrafish Lacking the Parkinson's Disease-Related Protein DJ-1 Mol. Neurobiol. 2019-06-19 [PMID: 31218647]

Porrini V, Mota M, Parrella E et al. Mild Inflammatory Profile without Gliosis in the c-Rel Deficient Mouse Modeling a Late-Onset Parkinsonism. Front Aging Neurosci 2017-08-03 [PMID: 28769786] (ICC/IF, Mouse)

Bonilha VL, Bell BA, Rayborn ME et al. Loss of DJ-1 elicits retinal abnormalities, visual dysfunction, and increased oxidative stress in mice Exp. Eye Res. 2015-07-26 [PMID: 26215528] (WB, Mouse)

Deng J, Zhao F, Yu X et al. Identification of the Protective Role of DJ-1 in Hypoglycemic Astrocyte Injury Using Proteomics. J Proteome Res 2015-06-19 [PMID: 26057206]

Choi MS, Nakamura T, Cho SJ et al. Transnitrosylation from DJ-1 to PTEN attenuates neuronal cell death in parkinson's disease models. J Neurosci 2014-11-05 [PMID: 25378175] (Human)

More publications at http://www.novusbio.com/NB300-270

Procedures

Western Blot Protocol for Park7/DJ-1 Antibody (NB300-270)
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.

Immunocytochemistry/Immunofluorescence Protocol for Park7/DJ-1 Antibody (NB300-270)
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.

Immunohistochemistry-Paraffin Protocol for Park7/DJ-1 Antibody (NB300-270)
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.

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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.

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