Product Datasheet

DRP1 Antibody
NB110-55288SS

Unit Size: 0.025 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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NB110-55288SS

DRP1 Antibody

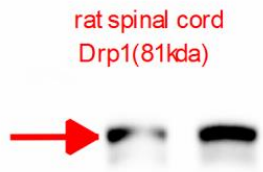
Product Information	
Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Immunogen affinity purified
Buffer	Tris-glycine, 150 mM NaCl
Target Molecular Weight	81 kDa

Product Description	
Host	Rabbit
Gene ID	10059
Gene Symbol	DNM1L
Species	Human, Mouse, Rat, Fish, Primate
Species Reactivity	Human, primate, rat, mouse. Predicted to react with bovine based on 100% sequence homology. Fish reactivity reported in scientific literature (PMID: 25008790)
Immunogen	A synthetic peptide made to an internal region within residues 500-600 of the human DRP1 protein. [Swiss-Prot# O00429]

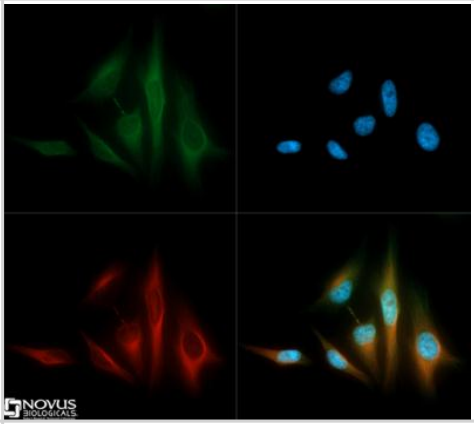
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Immunocytochemistry/Immunofluorescence 1:500, Immunohistochemistry 2.5 ug/ml, Immunohistochemistry-Paraffin 2.5 ug/ml, Western Blot 1:500, Immunoprecipitation, Simple Western 1:50
Application Notes	This DRP1 antibody is useful for Immonohistochemistry paraffin embedded sections, Immunocytochemistry/Immunofluorescence and Western blot analysis where a band is seen at ~81 kDa. In ICC/IF, cytoplasmic staining was observed in HeLa cells. Immunoprecipitation was reported in scientific literature.In Simple Western only 10-15 uL of the recommended dilution is used per data point.

Images

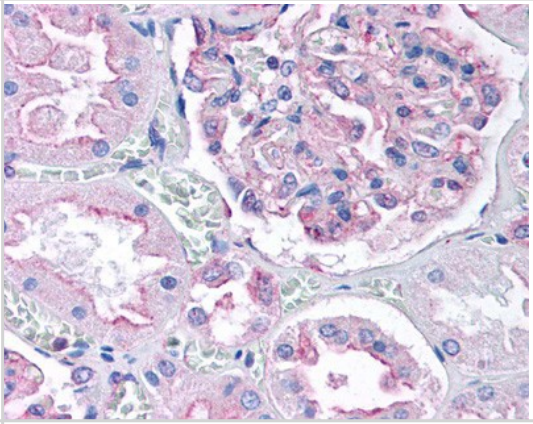
Western Blot: DRP1 Antibody [NB110-55288] - Rat spinal cord-DRP1 (81kda). Image from verified customer review.



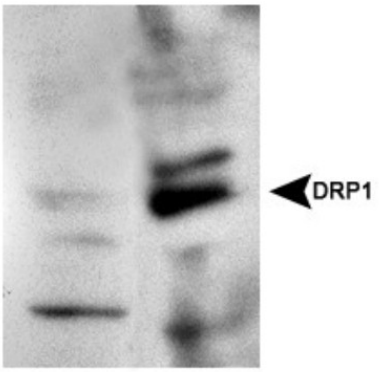
Immunocytochemistry/Immunofluorescence: DRP1 Antibody [NB110-55288] - DRP1 antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



Immunohistochemistry: DRP1 Antibody [NB110-55288] - Staining of renal tubular epithelium and visceral epithelial cells of the glomerulus. Human kidney cortex, 40X magnification.



Western Blot: DRP1 Antibody [NB110-55288] - Lane 1: DRP1 knockout Lane 2: DRP1 wildtype MEFs Stained with NB110-55288 at 1:500x dilution.



Simple Western: DRP1 Antibody [NB110-55288] - Simple Western lane view shows a specific band for DUX4 in 0.5 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

Publications

Su YC, Chiu HW, Hung JC, Hong JR. Beta-nodavirus B2 protein induces hydrogen peroxide production, leading to Drp1-recruited mitochondrial fragmentation and cell death via mitochondrial targeting. Apoptosis 2014 Jul 10 [PMID: 25008790] (WB, Fish)

Details:
DRP1 antibody used for WB at 1:500 dilution on mitochondrial fractions isolated from Grouper/Epinephelus coioides cell line GF-1 cells (a cell line susceptible to grouper nervous necrosis virus - GNNV) transfected with EYFP or EYFP-B2 and treated with and without NAC or Mdivi for 48 h (Fig 6C).

Amadoro G, Corsetti V, Florenzano F et al. AD-linked, toxic NH2 human tau affects the quality control of mitochondria in neurons. Neurobiol. Dis. 2014 Feb 1 [PMID: 24411077] (WB, Rat)

Kawada I, Hasina R, Lennon FE et al. Paxillin Mutations Affect Focal Adhesions and Lead to Altered Mitochondrial Dynamics: Relevance to Lung Cancer. Cancer Biol Ther 2013 May 31 [PMID: 23792636] (WB, IHC-P, Human)

Tseng AH, Shieh SS, Ling Wang D. SIRT3 deacetylates FOXO3 to protect mitochondria against oxidative damage. Free Radic Biol Med 2013 May 7 [PMID: 23665396] (WB, Human)

Bajpai P, Sangar MC, Singh S et al. Metabolism of 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine by Mitochondrion-targeted Cytochrome P450 2D6: IMPLICATIONS IN PARKINSON DISEASE. J Biol Chem 2013 Feb 8 [PMID: 23258538] (ICC/IF, Mouse)

Luzzo KM, Wang Q, Purcell SH et al. High fat diet induced developmental defects in the mouse: oocyte meiotic aneuploidy and fetal growth retardation/brain defects PLoS One 2012 [PMID: 23152876] (IHC, ICC/IF, Mouse)

Tang D, Kang R, Livesey KM et al. High-mobility group box 1 is essential for mitochondrial quality control Cell Metab 2011 Jun 8 [PMID: 21641551] (WB, Mouse)

Ferrari LF, Chum A, Bogen O et al. Role of drp1, a key mitochondrial fission protein, in neuropathic pain. J Neurosci;31(31):11404-11410. 2011 Aug 3. [PMID: 21813700]

Manczak M, Calkins MJ, Reddy PH. Impaired Mitochondrial Dynamics and Abnormal Interaction of Amyloid Beta with Mitochondrial Protein Drp1 in Neurons from Patients with Alzheimer's Disease: Implications for Neuronal Damage. Hum Mol Genet. 2011 Mar 31. [PMID: 21459773] (WB, IP, ICC/IF, Human)

Procedures

Western Blot Protocol for DRP1 Antibody (NB110-55288)
Western Blot Protocol

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 30 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH2O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS, 1 hour at room temperature.
6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-DRP1 primary antibody (NB 110-55288) in blocking buffer and incubate 2 hours at room temperature.
8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 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 manufacturers instructions (Pierce, ECL).

Note: Tween-20 can be added to the blocking or antibody diultion buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

IHC-FFPE sectionsI. 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.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 1-1.5 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).

Immunocytochemistry/Immunofluorescence Protocol for DRP1 Antibody (NB110-55288)
Immunocytochemistry Protocol

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Novus Biologicals USA
8100 Southpark Way, A-8
Littleton, CO 80120
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
novus@novusbio.com

Novus Biologicals Canada
461 North Service Road West, Unit B37
Oakville, ON L6M 2V5
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada@novusbio.com

Novus Biologicals Europe
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: technical@novusbio.com
Orders: orders@novusbio.com
General: novus@novusbio.com

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.