Product Datasheet

OPA1 Antibody
NB110-55290SS

Unit Size: 0.025 ml

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

Reviews: 1 Publications: 10

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB110-55290

Updated 6/15/2014 v.20.1

NB110-55290SS

OPA1 Antibody

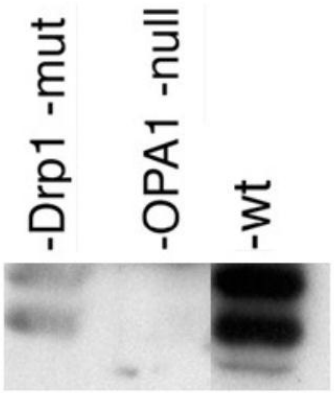
Product Information	
Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS
Target Molecular Weight	111 kDa

Product Description	
Host	Rabbit
Gene ID	4976
Gene Symbol	OPA1
Species	Human, Mouse, Rat, Chicken
Species Reactivity	Human, mouse, rat and chicken. Predicted to react with primate based on 100% sequence homology. Immunogen sequence has 94% homology to Zebrafish.
Immunogen	A synthetic peptide made to an internal region within residues 500-600 of human OPA1. [Swiss-Prot# O60313]

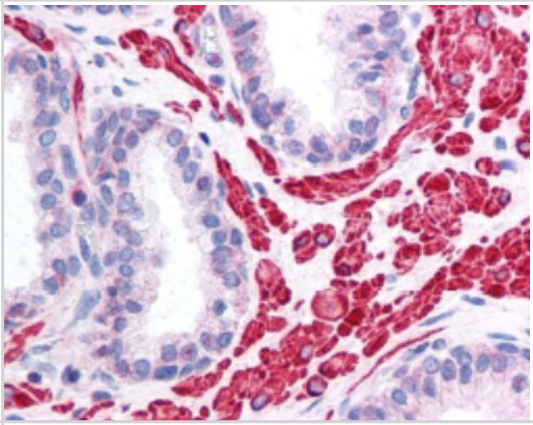
Product Application Details	
Applications	Western Blot, Simple Western, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Immunohistochemistry 2.5 ug/ml, Immunohistochemistry-Paraffin 2.5 ug/ml, Western Blot 2 ug/ml, Simple Western 20 ug/ml
Application Notes	This OPA1 antibody is useful for Immunohistochemistry on paraffin-embedded sections and Western blot analysis, where a band is seen at ~111 kDa.In Simple Western only 10-15 uL of the recommended dilution is used per data point.

Images

Western Blot: OPA1 Antibody [NB110-55290] - Detection of OPA1 protein in post-nuclear extracts of mouse embryonic fibroblasts.



Immunohistochemistry: OPA1 Antibody [NB110-55290] - Staining in prostatic smooth muscle and glandular epithelium. Human Prostate 40X magnification.



Simple Western: OPA1 Antibody [NB110-55290] - Simple Western lane view shows a specific band for OPA1 in 0.5 mg/ml of MEF lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

Publications

Lee KP, Jin Shin Y, Chun Cho S et al. Peroxiredoxin 3 has a Crucial Role in the Contractile Function of Skeletal Muscle via Regulating Mitochondrial Homeostasis. Free Radic. Biol. Med. 2014 Sep 12 [PMID: 25224038]

Lionetti L, Mollica MP, Donizzetti I et al. High-lard and high-fish-oil diets differ in their effects on function and dynamic behaviour of rat hepatic mitochondria. PLoS ONE 3/25/2014 [PMID: 24663492] (WB, IHC-P, Rat)

Hara H, Araya J, Ito S et al. Mitochondrial fragmentation in cigarette smoke-induced bronchial epithelial cell senescence. Am J Physiol Lung Cell Mol Physiol. 2013 Nov [PMID: 24056969] (WB, Human)

Ussakli CH, Ebaee A, Binkley J et al. Mitochondria and Tumor Progression in Ulcerative Colitis. J Natl Cancer Inst 2013 Jul 12 [PMID: 23852949] (IHC, Human)

Rahn JJ, Stackley KD, Chan SS. Opa1 is required for proper mitochondrial metabolism in early development PLoS One 2013 [PMID: 23516612] (WB, Zebrafish)

Trushina E, Nemutlu E, Zhang S et al. Defects in Mitochondrial Dynamics and Metabolomic Signatures of Evolving Energetic Stress in Mouse Models of Familial Alzheimer's Disease PLoS ONE 2012 [PMID: 22393443] (WB, Mouse)

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

Johnson D, Allman E, Nehrke K. Regulation of acid-base transporters by reactive oxygen species following mitochondrial fragmentation. Am J Physiol Cell Physiol;302(7):C1045-54. 2012 Apr. [PMID: 22237403] (WB, Rat)

Dai DF, Hsieh EJ, Liu Y, Chen T, Beyer RP, Chin MT, MacCoss MJ, Rabinovitch PS. Mitochondrial proteome remodelling in pressure overload-induced heart failure: the role of mitochondrial oxidative stress. Cardiovasc Res;93 (1):79-88. 2012 Jan 1. [PMID: 22012956] (WB, Mouse)

Vallon M, Aubele P, Janssen KP, Essler M. Thrombin-induced shedding of tumor endothelial marker 5 and exposure of its RGD motif are regulated by cell surface protein disulfide isomerase. Biochem J. 2011 Oct 20. [PMID: 22013897] (WB, Human)

Procedures

Protocol specific for OPA1 Antibody (NB110-55290)
Western Blot Protocol

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 32 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-Opa1 primary antibody (NB110-55290) 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 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. 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).

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.