

# Product Datasheet

## OPA1 Antibody - BSA Free

### NB110-55290

Unit Size: 0.1 ml

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

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**NB110-55290**

OPA1 Antibody - BSA Free

Product Information	
<b>Unit Size</b>	0.1 ml
<b>Concentration</b>	1 mg/ml
<b>Storage</b>	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
<b>Clonality</b>	Polyclonal
<b>Preservative</b>	0.02% Sodium Azide
<b>Isotype</b>	IgG
<b>Purity</b>	Immunogen affinity purified
<b>Buffer</b>	PBS
<b>Target Molecular Weight</b>	111 kDa

Product Description	
<b>Description</b>	Novus Biologicals Rabbit OPA1 Antibody - BSA Free (NB110-55290) is a polyclonal antibody validated for use in IHC, WB, ICC/IF and Simple Western. Anti-OPA1 Antibody: Cited in 48 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
<b>Host</b>	Rabbit
<b>Gene ID</b>	4976
<b>Gene Symbol</b>	OPA1
<b>Species</b>	Human, Mouse, Rat, Porcine, Chicken, Zebrafish
<b>Reactivity Notes</b>	Zebrafish reactivity reported in scientific literature (PMID: 23516612, 26365306).
<b>Immunogen</b>	A synthetic peptide made to an internal region within residues 500-600 of human OPA1. [Swiss-Prot: O60313]

Product Application Details	
<b>Applications</b>	Western Blot, Simple Western, Immunohistochemistry-Paraffin, Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry
<b>Recommended Dilutions</b>	Western Blot 2 ug/ml, Simple Western 20 ug/ml, Immunohistochemistry 2.5 ug/ml, Immunocytochemistry/ Immunofluorescence reported in scientific literature (PMID 34923139), Immunohistochemistry-Paraffin 2.5 ug/ml, Immunoblotting reported in scientific literature (PMID 25224038)
<b>Application Notes</b>	In Western blot, a band is seen at approx. 111 kDa. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See <a href="#">Simple Western Antibody Database</a> for Simple Western validation: antibody dilution of 20 ug/mL

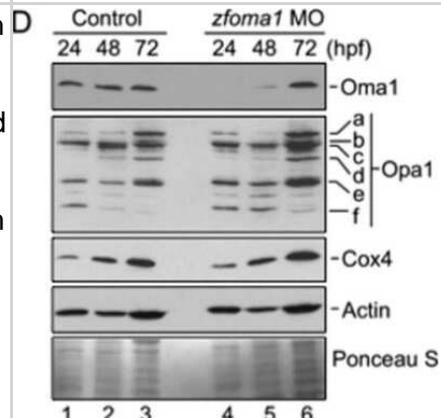


## Images

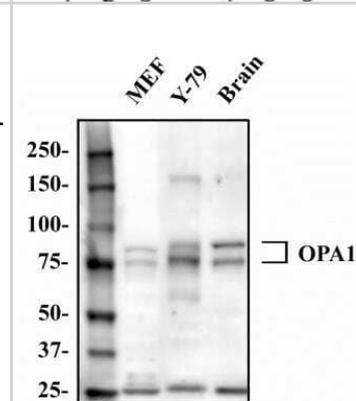
Simple Western: OPA1 Antibody [NB110-55290] - Lane view shows a specific band for OPA1 in 0.5 mg/ml of MEF lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



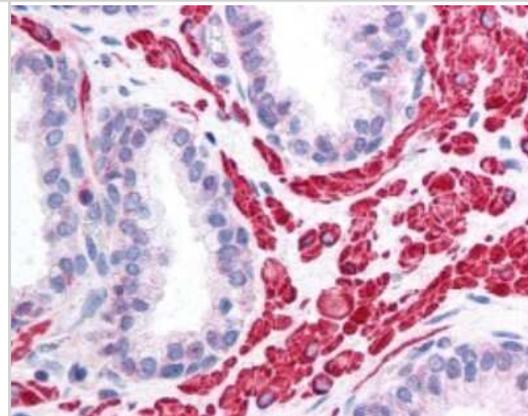
Western Blot: OPA1 Antibody [NB110-55290] - Depletion of Oma1 in fish affects development. Immunoblot of protein extracts from injected fish (yolk removed) at 24, 48 and 72 hpf. Expression of Oma1, Opa1 isoforms (a-f) and Cox4 (mitochondrial abundance marker) was analyzed with respective antibodies. Actin and Ponceau S staining were loading controls. Source data (full-length blots) are available online in Supplementary information. Image collected and cropped by CiteAb from the following publication (<https://www.nature.com/articles/srep13989>), licensed under a CC-BY license.



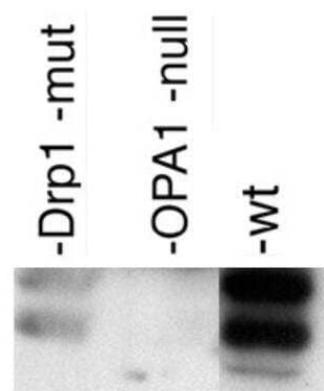
Western Blot: OPA1 Antibody [NB110-55290] - Total protein from Human HeLa cells, Mouse MEF cells and Rat Brain was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-OPA1 in 1% blocking buffer. Precision Plus Protein All Blue molecular weight markers (BioRad) were detected with 1 ug/ml Anti-Blue Marker antibody (NBP2-33376). The protein ladder was detected with an anti-mouse HRP secondary antibody and OPA1 with an anti-rabbit HRP secondary antibody using chemiluminescence.



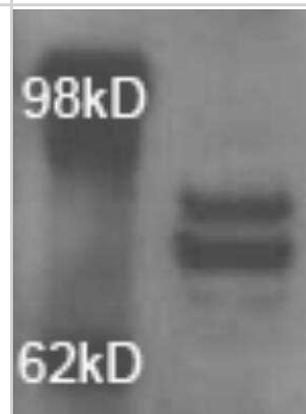
Immunohistochemistry: OPA1 Antibody [NB110-55290] - Staining in prostatic smooth muscle and glandular epithelium. Human Prostate 40X magnification.



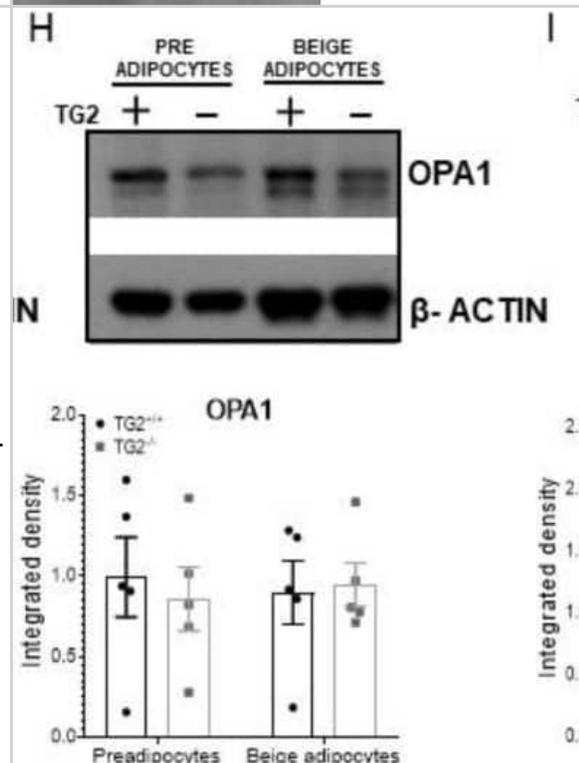
Western Blot: OPA1 Antibody [NB110-55290] - Detection of OPA1 protein in post-nuclear extracts of mouse embryonic fibroblasts.



Western Blot: OPA1 Antibody [NB110-55290] - Analysis of OPA1 in porcine retinal pigment epithelium lysate using anti-OPA1 antibody. Image from verified customer review.



Western Blot: OPA1 Antibody - BSA Free [NB110-55290] - High content screening of the preadipocytes & beige cells, & detection of mitochondrial fission- & fusion-related proteins: (A) Representative high content screening images showing mitochondrial morphology in TG2<sup>+/+</sup> & TG2<sup>-/-</sup> preadipocytes, scale bars represent 50  $\mu$ m; (B) fractions of fragmented & tubular mitochondrial morphology in preadipocytes (%); (C) quantitative analysis of mitochondrial morphology in preadipocytes; (D) representative high content screening images showing mitochondrial morphology in TG2<sup>+/+</sup> & TG2<sup>-/-</sup> beige cells; (E) fractions of fragmented & tubular mitochondrial morphology in beige cells (%); (F) quantitative analysis of mitochondrial morphology in beige cells, DAPI staining was used to determine the number of nuclei, the mitochondria were stained with Mito Tracker Deep Red either in the absence or presence of 10  $\mu$ M antimycin A, Texas Red-X phalloidin was used to stain actin filaments for the detection of cell shapes (n = 3); (G–J) representative Western blot analyses & quantitative analyses of the mitochondrial fusion proteins (MFN2, OPA1) & mitochondrial fission proteins (DRP1, MFF) in preadipocytes & differentiated beige cells.  $\beta$ -ACTIN was used as a loading control. Columns represent the mean values  $\pm$  SD. Statistical analyses were performed using Student's t-test. n = 5. \* p < 0.05. Image collected & cropped by CiteAb from the following publication (<https://pubmed.ncbi.nlm.nih.gov/35563567>), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



## Publications

Douida A, Batista F, Boto P et al. Cells Lacking PA200 Adapt to Mitochondrial Dysfunction by Enhancing Glycolysis via Distinct Opa1 Processing International Journal of Molecular Sciences 2021-02-05 [PMID: 33562813] (Western Blot, Mouse)

Soundararajan R, Hernandez-Cuervo H, Stearns TM et al. A-Kinase Anchor Protein 1 deficiency causes mitochondrial dysfunction in mouse model of hyperoxia induced acute lung injury Frontiers in Pharmacology 2022-10-03 [PMID: 36263130] (Western Blot, Mouse)

Lönert K, Bank C, Ujlaki G et al. Tissue Transglutaminase Knock-Out Preadipocytes and Beige Cells of Epididymal Fat Origin Possess Decreased Mitochondrial Functions Required for Thermogenesis International Journal of Molecular Sciences 2022-05-05 [PMID: 35563567] (Western Blot, Mouse)

Faiq MA, Sengupta T, Nath M et al. Ocular manifestations of central insulin resistance Neural Regeneration Research 2023-01-01 [PMID: 36255004] (Western Blot, Mouse)

Meng Q, Zaharieva EK, Sasatani M, Kobayashi J. Possible relationship between mitochondrial changes and oxidative stress under low dose-rate irradiation Redox Report 2021-08-26 [PMID: 34435550] (Western Blot, Mouse)

G Li, YL Chan, S Sukjamnong, AG Anwer, H Vindin, M Padula, R Zakarya, J George, BG Oliver, S Saad, H Chen A Mitochondrial Specific Antioxidant Reverses Metabolic Dysfunction and Fatty Liver Induced by Maternal Cigarette Smoke in Mice Nutrients, 2019-07-21;11(7):. 2019-07-21 [PMID: 31330878] (Western Blot, Mouse)

Wongkitkamjorn W, Hosomichi J, Wada E et al. Gestational Intermittent Hypoxia Induces Mitochondrial Impairment in the Geniohyoid Muscle of Offspring Rats Cureus 2022-05-17 [PMID: 35600069] (Western Blot, Mouse)

Senthil K, Ranganathan A, Piel S et al. Elevated serum neurologic biomarker profiles after cardiac arrest in a porcine model. Resuscitation plus 2024-09-01 [PMID: 39149222]

Mariacristina Filice, Alfonsina Gattuso, Sandra Imbrogno, Rosa Mazza, Daniela Amelio, Alessia Caferro, Claudio Agnisola, José Manuel Icardo, Maria Carmela Cerra Functional, structural, and molecular remodelling of the goldfish (Carassius auratus) heart under moderate hypoxia Fish Physiology and Biochemistry 2024-01-10 [PMID: 38198074]

Philipp Portz, Michael K. Lee, Ilana Chafetz Menaker Changes in Drp1 Function and Mitochondrial Morphology Are Associated with the  $\alpha$ -Synuclein Pathology in a Transgenic Mouse Model of Parkinson's Disease Cells 2021-04-13 [PMID: 33924585]

Saito ER, Warren CE, Hanegan CM et al. A Novel Ketone-Supplemented Diet Improves Recognition Memory and Hippocampal Mitochondrial Efficiency in Healthy Adult Mice Metabolites 2022-10-25 [PMID: 36355101] (WB, Mouse)

Wang D, Kuang Y, Wan Z et al. Aspartate Alleviates Colonic Epithelial Damage by Regulating Intestinal Stem Cell Proliferation and Differentiation via Mitochondrial Dynamics Molecular nutrition & food research 2022-10-30 [PMID: 36310136] (WB)

More publications at <http://www.novusbio.com/NB110-55290>

## Procedures

### Serum protocol 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 dH<sub>2</sub>O 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 dH<sub>2</sub>O 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 dH<sub>2</sub>O 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 dilution 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).



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### **Products Related to NB110-55290**

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NB820-59177	Human Brain Whole Tissue Lysate (Adult Whole Normal)
NB110-55290PEP	OPA1 Antibody Blocking Peptide
NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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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