Product Datasheet

COX4 Antibody - BSA Free
NB110-39115

Unit Size: 0.1 ml

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

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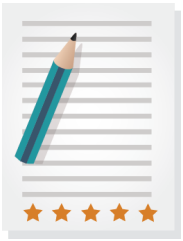
Updated 12/20/2023 v.20.1



NB110-39115

COX4 Antibody - BSA Free

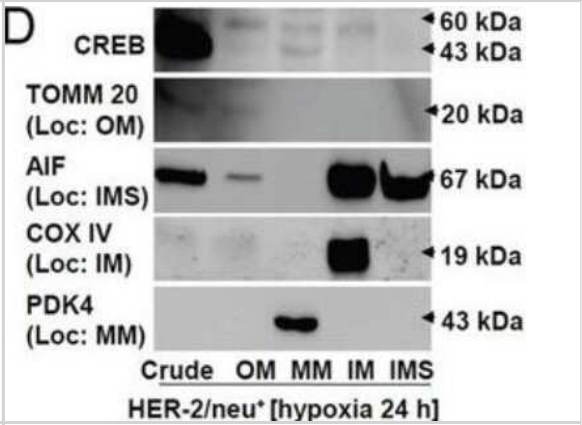
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.02% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS

Product Description	
Host	Rabbit
Gene ID	1327
Gene Symbol	COX4I1
Species	Human, Mouse, Rat, Porcine, Bovine, Drosophila, Insect, Opossum, Primate
Reactivity Notes	Opossum reactivity reported in scientific literature (PMID: 28720662).
Marker	Mitochondria Marker
Immunogen	A synthetic peptide made to an internal region of human COX IV isoform 1 (within residues 1-100). [Swiss-Prot# P13073]

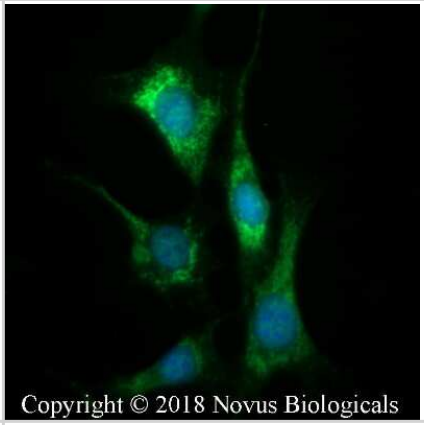
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Chromatin Immunoprecipitation (ChIP), Immunocytochemistry, Knockdown Validated
Recommended Dilutions	Western Blot 1:2000, Simple Western 1:25, Immunohistochemistry 1:100, Immunocytochemistry/ Immunofluorescence 1:40, Immunohistochemistry-Paraffin 1:100, Chromatin Immunoprecipitation (ChIP), Immunocytochemistry, Knockdown Validated reported in scientific literature (PMID 31655343)
Application Notes	In Western Blot, a band is seen ~19.5 kDa representing COX IV. In ICC/IF, mitochondrion staining was observed in HeLa cells. In IHC-P, staining is observed in the cytoplasm and mitochondria of human breast cancer tissue. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended. Higher dilutions may be needed for mitochondrial membrane enriched preparations. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.

Images

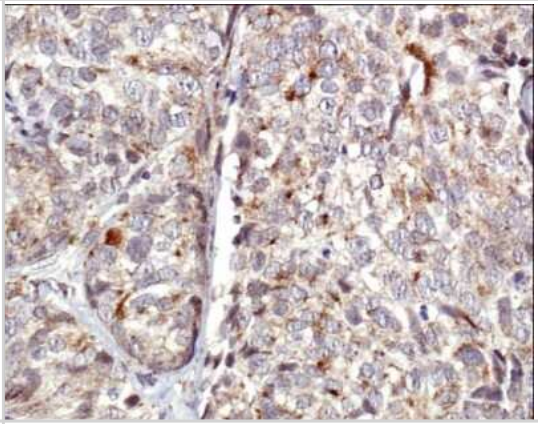
Western Blot: COX4 Antibody - BSA Free [NB110-39115] - Increased mitochondrial localization of CREB under hypoxiaA. Mitochondria were sub fractionated into outer membrane (OM), inter membrane space (IMS), inner membrane (IM) and mitochondrial matrix (MM). The purity of the fractions was analysed with marker proteins: TOM20 for OM, AIF for IMS, COX4 for IM, PDK4 for MM. Image collected and cropped by CiteAb from the following publication (https://www.oncotarget.com/article/10474/text/) licensed under a CC-BY license.



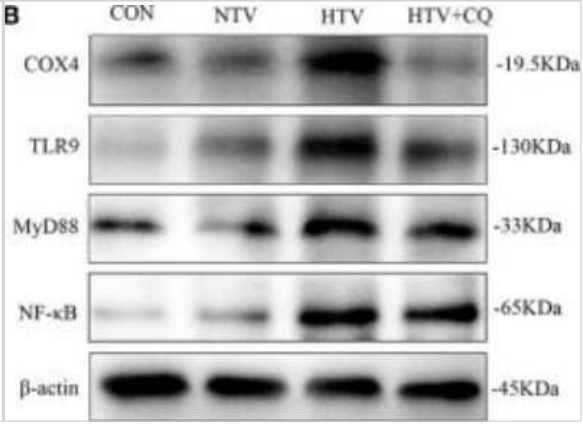
Immunocytochemistry/Immunofluorescence: COX4 Antibody - BSA Free [NB110-39115] - NIH3T3 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton X-100. The cells were incubated with anti-COX4 at 10 ug/mL overnight at 4C and detected with an anti-rabbit DyLight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

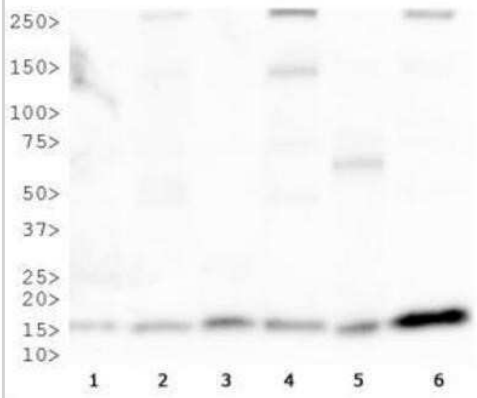
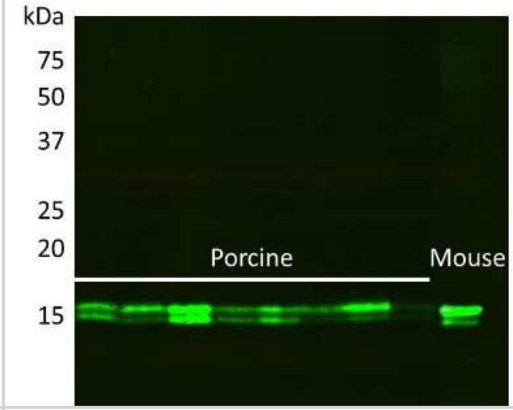
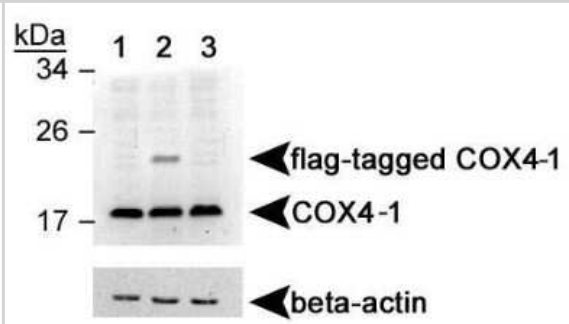
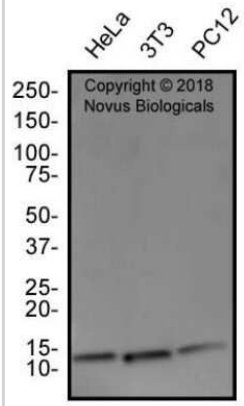


Immunohistochemistry: COX4 Antibody - BSA Free [NB110-39115] - Analysis of COX4 in human breast cancer using DAB with hematoxylin counterstain.

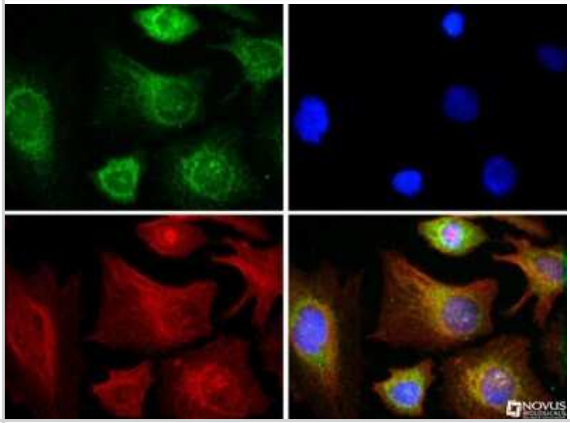


Western Blot: COX4 Antibody - BSA Free [NB110-39115] - The mRNA and protein expression of cytochrome c oxidase 4 (COX4), toll-like receptor (TLR) 9, myeloid differentiation factor 88 (MyD88), and nuclear factor (NF)-kB in lung tissues from spontaneous breathing group (CON group), normal tidal volume (NTV) group, high tidal volume (HTV) group, chloroquine (CQ) pretreatment upon HTV stimulation group (HTV + CQ). Levels of COX4, TLR9, MyD88, and NF-kB protein by Western blot. Fold expression for target genes was normalized to that measured for the beta-actin gene. Both of these experiments were in triplicate. Image collected and cropped by CiteAb from the following publication (//pubmed.ncbi.nlm.nih.gov/30018615/) licensed under a CC-BY license.

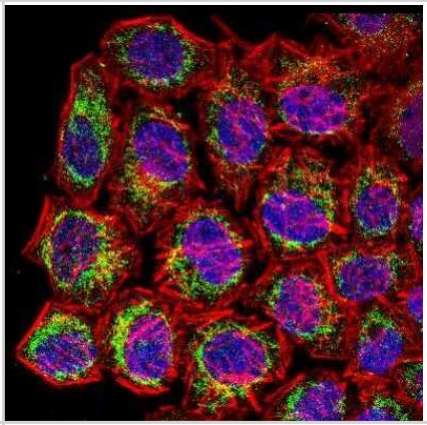


Western Blot: COX4 Antibody - BSA Free [NB110-39115] - Analysis of COX4 in the following cell lysates: 1. HeLa, 2. Ntera, 3. A431, 4. HepG2, 5. MCF7 and 6. 3T3.	
Western Blot: COX4 Antibody - BSA Free [NB110-39115] - Analysis of COX4 in porcine whole skeletal muscle lysate using anti-COX4 antibody. Image from verified customer review.	
Western Blot: COX4 Antibody - BSA Free [NB110-39115] - Detection of COX4-1 isoform using COX4 antibody NB110-39115. Lane 1: HEK 293 with empty vector, Lane 2: HEK 293 with flag-tagged human COX4-1, Lane 3: HEK 293 with flag-tagged human COX4-2. Photo courtesy of Ryo Fukuda, G. Semenza lab. Johns Hopkins, SOM	
Western Blot: COX4 Antibody - BSA Free [NB110-39115] - Total protein from HeLa, 3T3 and PC12 was separated on a 4-20% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 0.5 ug/mL anti-COX4 in 5% block buffer and detected with an anti-rabbit HRP secondary antibody using chemiluminescence.	

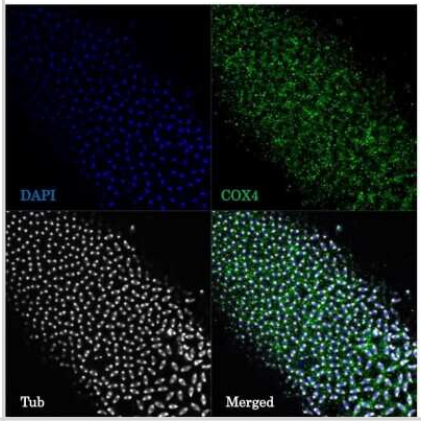
Immunocytochemistry/Immunofluorescence: COX4 Antibody - BSA Free [NB110-39115] - COX4 antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



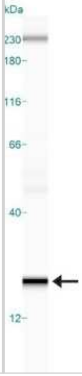
Immunocytochemistry/Immunofluorescence: COX4 Antibody - BSA Free [NB110-39115] - Analysis of HeLa cells using COX4 antibody (NB110-39115, 1:5). An Alexa Fluor 488-conjugated Goat to rabbit IgG was used as secondary antibody (green). Actin filaments were labeled with Alexa Fluor 568 phalloidin (red). DAPI was used to stain the cell nuclei (blue).



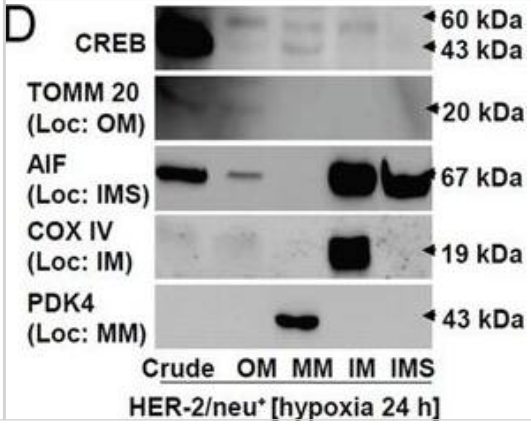
Immunocytochemistry: COX4 Antibody - BSA Free [NB110-39115] - Analysis of methanol fixed drosophila embryo using 1:200 dilution of COX4 antibody. The signal was developed using AF488 conjugated Donkey anti-Rabbit IgG (H+L) secondary antibody and the sections were further counterstained for tubulin and DAPI. The antibody generated a specific staining of COX4 in mitochondria near mitotic spindles at early stage of embryo development. Image from verified customer review.



Simple Western: COX4 Antibody - BSA Free [NB110-39115] - Image shows a specific band for COX4 in 1.0 mg/mL of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.



Small interference RNA targeting Raptor and Rictor disrupts BMAL1 accumulation in HNSCCTargeted disruption of Raptor (A-B) and Rictor (C-D) using siRNA results in a dose-dependent downregulation of BMAL1 in HNSCC cells. E. Disruption of PTEN by protein oxidation causes activation of mTOR signaling, resulting in accumulation of BMAL1. Notably, inhibition of mTOR signaling, particularly mTORC1 and mTORC2, results in restoration of normal BMAL1 levels in the epidermis of mice and head and neck cancer cells. These results demonstrate a novel role for mTOR in regulating nuclear levels of the core clock gene BMAL1. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/27285754), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Zhao Y, Liu Y, Zhao G et al. Myeloid BAF60a deficiency alters metabolic homeostasis and exacerbates atherosclerosis Cell reports 2023-09-26 [PMID: 37768825] (WB, Mouse)

Papadaki V, Erpapazoglou Z, Kokkori M et al. IQGAP1 mediates the communication between the nucleus and the mitochondria via NDUFS4 alternative splicing NAR Cancer 2023-08-24 [PMID: 37636315]

Madhu V, Boneski PK, Silagi E et al. Hypoxic Regulation of Mitochondrial Metabolism and Mitophagy in Nucleus Pulposus Cells Is Dependent on HIF-1?-BNIP3 Axis Journal of Bone and Mineral Research 2020-08-01 [PMID: 32251541] (WB)

Chung YJ, Swietach P, Curtis MK et al. Iron-Deficiency Anemia Results in Transcriptional and Metabolic Remodeling in the Heart Toward a Glycolytic Phenotype Frontiers in Cardiovascular Medicine 2021-01-21 [PMID: 33553263] (In Vivo)

Jing X, Wu J, Dong C et al. COVID-19 instigates adipose browning and atrophy through VEGF in small mammals Nature metabolism 2022-12-01 [PMID: 36482111] (IHC-P, Mouse)

Kaseder M, Schmid N, Eubler K et al. Evidence of a role for cAMP in mitochondrial regulation in ovarian granulosa cells Molecular human reproduction 2022-08-09 [PMID: 35944223]

Treidel LA, Quintanilla Ramirez GS, Chung DJ et al. Selection on dispersal drives evolution of metabolic capacities for energy production in female wing-polymorphic sand field crickets, Gryllus firmus Journal of evolutionary biology 2022-03-07 [PMID: 35255175] (WB, Insect)

Details:
Gryllus firmus

Burmakin M, Fasching A, Kobayashi H et al. Pharmacological HIF-PHD inhibition reduces renovascular resistance and increases glomerular filtration by stimulating nitric oxide generation Acta physiologica (Oxford, England) 2021-04-26 [PMID: 33900001] (IF/IHC, Rat)

Moskal N, Riccio V, Bashkurov M et al. ROCK inhibitors upregulate the neuroprotective Parkin-mediated mitophagy pathway Nat Commun 2020-01-03 [PMID: 31900402]

Huang CY, Hsu LH, Chen CY et al. Inhibition of Alternative Cancer Cell Metabolism of EGFR Mutated Non-Small Cell Lung Cancer Serves as a Potential Therapeutic Strategy Cancers (Basel) 2020-01-10 [PMID: 31936895] (WB, Human)

Long KR, Shipman KE, Rbaibi Y et al. Proximal tubule apical endocytosis is modulated by fluid shear stress via an mTOR-dependent pathway Mol Biol Cell. 2017-09-14 [PMID: 28720662] (WB, Opossum)

Du XK, Ge WY, Jing R, Pan LH Necroptosis in pulmonary macrophages mediates lipopolysaccharide-induced lung inflammatory injury by activating ZBP-1 Int. Immunopharmacol. 2019-10-23 [PMID: 31655343] (WB, KD, Mouse)

More publications at http://www.novusbio.com/NB110-39115

Procedures

Western Blot Protocol specific for COX IV isoform 1 Antibody (NB110-39115)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted 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 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 manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

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

Immunohistochemistry-Paraffin Embedded Sections Protocol specific for COX IV isoform 1 Antibody (NB110-39115)

Immunohistochemistry-Paraffin Embedded Sections Protocol

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.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution 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 biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

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Immunocytochemistry/Immunofluorescence Protocol for COX IV Antibody (NB110-39115)

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.

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Products Related to NB110-39115

NB800-PC1	HeLa Whole Cell Lysate
NB110-39115PEP	COX4 Antibody Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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