Product Datasheet

COX4 Antibody
NB110-39115SS

Unit Size: 0.025 ml

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

Reviews: 1 Publications: 2

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NB110-39115SS

COX4 Antibody

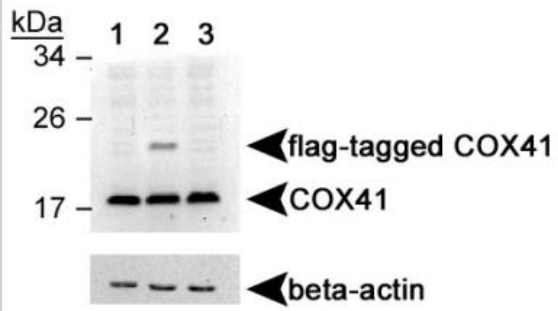
Product Information	
Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Immunogen affinity purified
Buffer	Tris-glycine, 150 mM NaCl

Product Description	
Host	Rabbit
Gene ID	1327
Gene Symbol	COX4I1
Species	Human, Mouse, Rat, Bovine, Primate
Species Reactivity	Human, mouse, bovine, primate and rat. Immunogen sequence has 76% homology to Zebrafish.
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]

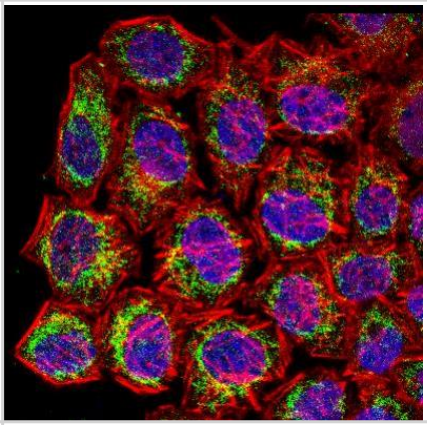
Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Immunocytochemistry/Immunofluorescence 1:40, Immunohistochemistry 1:100, Immunohistochemistry-Paraffin 1:100, Western Blot 1:2000, Simple Western 1:25
Application Notes	This COX IV isoform 1 antibody is useful for Western Blot, Immunocytochemistry/Immunofluorescence, and Immunohistochemistry paraffin embedded sections. 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.

Images

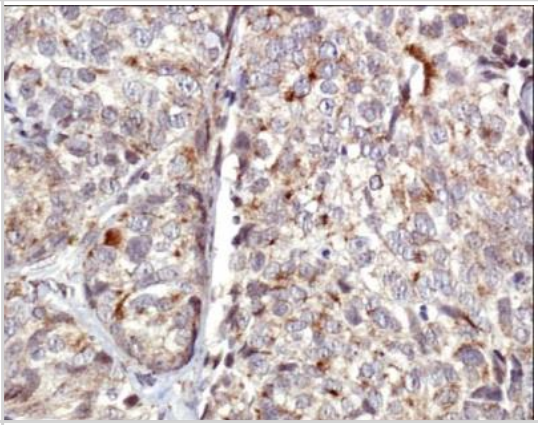
Western Blot: COX4 Antibody [NB110-39115] - Detection of COX41 using NB110-39115. Lane 1: HEK 293 with empty vector. Lane 2: HEK 293 with flag-tagged human COX4. Lane 3: HEK 293 with flag-tagged human COX42. Photo courtesy of Ryo Fukuda, G. Semenza lab. Johns Hopkins, SOM



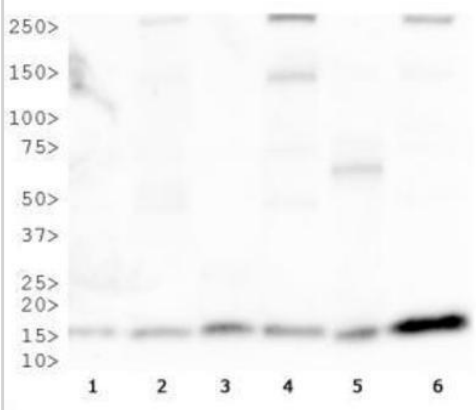
Immunocytochemistry/Immunofluorescence: COX4 Antibody [NB110-39115] - IF confocal analysis of HeLa cells using COX4 antibody (NB110 -39115, 1:5). An Alexa Fluor 488-conjugated Goat to rabbit IgG was used as secondary antibody (green). Actin filaments were labeled with Alexa Fluor 568 phalloidin (red).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

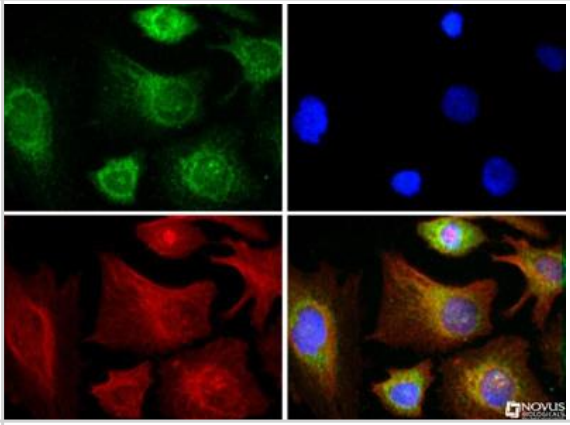


Immunohistochemistry: COX4 Antibody [NB110-39115] - IHC analysis of COX4 in human breast cancer using DAB with hematoxylin counterstain.



Western Blot: COX4 Antibody [NB110-39115] - WB analysis of COX4 in the following cell lysates: 1. HeLa, 2. Ntera, 3. A431, 4. HepG2, 5. MCF7 and 6. 3T3.



Immunocytochemistry/Immunofluorescence: COX4 Antibody [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).	
Simple Western: COX4 Antibody [NB110-39115] - Simple Western lane view 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.	

Publications

Zan H, Zhang J, Al-Qahtani A et al. Endonuclease G plays a role in immunoglobulin class switch DNA recombination by introducing double-strand breaks in switch regions. Mol Immunol 2011 Jan [PMID: 21111482] (WB, Human, Mouse)

Morten KJ, Badder L, Knowles HJ. Differential regulation of HIF-mediated pathways increases mitochondrial metabolism and ATP production in hypoxic osteoclasts J Pathol 2013 Apr [PMID: 23303559] (WB, Human)

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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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.