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Anti-COX IV/COX4I1 Antibody Picoband™

Catalog Number: A05442-1

About COX4I1

Cytochrome c oxidase subunit 4 isoform 1, mitochondrial is an enzyme that in humans is encoded by the COX4I1 gene. Cytochrome c oxidase (COX) is the terminal enzyme of the mitochondrial respiratory chain. It is a multi-subunit enzyme complex that couples the transfer of electrons from cytochrome c to molecular oxygen and contributes to a proton electrochemical gradient across the inner mitochondrial membrane. The complex consists of 13 mitochondrial- and nuclear-encoded subunits. The mitochondrially-encoded subunits perform the electron transfer and proton pumping activities. The functions of the nuclear-encoded subunits are unknown but they may play a role in the regulation and assembly of the complex. This gene encodes the nuclear-encoded subunit IV isoform 1 of the human mitochondrial respiratory chain enzyme. It is located at the 3' of the NOC4 (neighbor of COX4) gene in a head-to-head orientation, and shares a promoter with it. Pseudogenes related to this gene are located on chromosomes 13 and 14.

Overview

Product Name	Anti-COX IV/COX4I1 Antibody Picoband™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-COX IV/COX4I1 Antibody Picoband [™] catalog # A05442-1. Tested in ELISA, Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	ELISA, Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na $_2$ HPO $_4$, 0.05mg NaN $_3$.
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	P13073

Technical Details

Immunogen	E. coli-derived human COX IV recombinant protein (Position: Q59-K169).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized



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Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: Western blot,0.1-0.5ug/ml Immunohistochemistry (Paraffin-embedded Section),0.5-1ug/ml Immunocytochemistry/Immunofluorescence, 2ug/ml Flow Cytometry, 1-3ug/1x10 ⁶ cells Direct ELISA,0.1-0.5ug/ml



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Anti-COX IV/COX4I1 Antibody Picoband[™] (A05442-1) Images

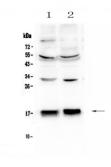


Figure 1. Western blot analysis of COX IV using anti-COX IV antibody (A05442-1).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: human HepG2 whole cell lysates, Lane 2: human SGC-7901 whole cell lysates. After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-COX IV antigen affinity purified polyclonal antibody (Catalog # A05442-1) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for COX IV at approximately 17KD. The expected band size for COX IV is at 20KD.

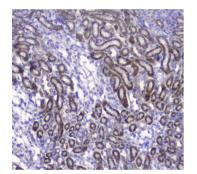


Figure 10. IHC analysis of COX IV using anti-COX IV antibody (A05442-1).

COX IV was detected in paraffin-embedded section of mouse kidney tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-COX IV Antibody (A05442-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.



Figure 2. Western blot analysis of COX IV using anti-COX IV antibody (A05442-1).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: rat heart tissue lysates,

Lane 2: mouse heart tissue lysates.

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-COX IV antigen affinity purified polyclonal antibody (Catalog # A05442-1) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection



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(ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for COX IV at approximately 17KD. The expected band size for COX IV is at 20KD.

Figure 3. IHC analysis of COX IV using anti-COX IV antibody (A05442-1).

COX IV was detected in paraffin-embedded section of mouse small intestine tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-COX IV Antibody (A05442-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

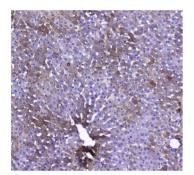


Figure 4. IHC analysis of COX IV using anti-COX IV antibody (A05442-1).

COX IV was detected in paraffin-embedded section of rat liver tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-COX IV Antibody (A05442-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

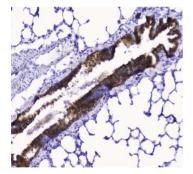


Figure 5. IHC analysis of COX IV using anti-COX IV antibody (A05442-1).

COX IV was detected in paraffin-embedded section of rat lung tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-COX IV Antibody (A05442-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

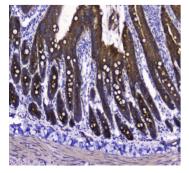


Figure 6. IHC analysis of COX IV using anti-COX IV antibody (A05442-1).

COX IV was detected in paraffin-embedded section of rat small intestine tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-COX IV Antibody (A05442-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue



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section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

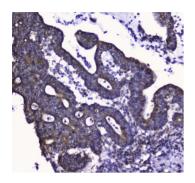


Figure 7. IHC analysis of COX IV using anti-COX IV antibody (A05442-1).

COX IV was detected in paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-COX IV Antibody (A05442-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

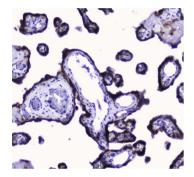


Figure 8. IHC analysis of COX IV using anti-COX IV antibody (A05442-1).

COX IV was detected in paraffin-embedded section of human placenta tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-COX IV Antibody (A05442-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

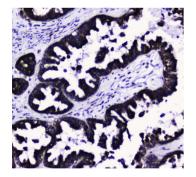


Figure 9. IHC analysis of COX IV using anti-COX IV antibody (A05442-1).

COX IV was detected in paraffin-embedded section of human thyroid cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2ug/ml rabbit anti-COX IV Antibody (A05442-1) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

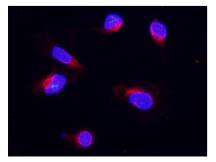


Figure 11. IF analysis of COX IV using anti-COX IV antibody (A05442-1).

COX IV was detected in immunocytochemical section of U20S cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/mL rabbit anti-COX IV Antibody (A05442-1) overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG (BA1032) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The



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section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

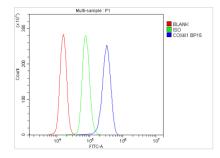


Figure 12. Flow Cytometry analysis of U937 cells using anti-COX IV antibody (A05442-1). Overlay histogram showing U937 cells stained with A05442-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-COX IV Antibody (A05442-1,1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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