

Anti-COX IV COX4I1 Antibody Picoband™ (monoclonal, 4G11)

Catalog # ABO14864

Product Information

Application	WB, IHC, FC
Primary Accession	P13073
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human, Mouse
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-COX IV COX4I1 Antibody Picoband™ (monoclonal, 4G11) . Tested in Flow Cytometry, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500 µg/ml.

Additional Information

Gene ID	1327
Other Names	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial, Cytochrome c oxidase polypeptide IV, Cytochrome c oxidase subunit IV isoform 1, COX IV-1, COX4I1 (HGNC:2265)
Calculated MW	19577
Application Details	Western blot, 0.1-0.5 µg/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml Flow Cytometry, 1-3 µg/1x10 ⁶ cells
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
Clone Names	Clone: 4G11
Immunogen	E. coli-derived human COX IV recombinant protein (Position: Q59-K169).
Cross Reactivity	No cross-reactivity with other proteins.
Storage	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.

Protein Information

Name	COX4I1 (HGNC:2265)
Function	Component of the cytochrome c oxidase, the last enzyme in the mitochondrial electron transport chain which drives oxidative phosphorylation. The respiratory chain contains 3 multisubunit complexes succinate dehydrogenase (complex II, CII), ubiquinol- cytochrome c oxidoreductase (cytochrome b-c1 complex, complex III, CIII) and cytochrome c oxidase (complex IV, CIV), that cooperate to transfer electrons derived from NADH and succinate to molecular oxygen, creating an electrochemical gradient over the inner membrane that drives transmembrane transport and the ATP synthase. Cytochrome c oxidase is the component of the respiratory chain that catalyzes the reduction of oxygen to water. Electrons originating from reduced cytochrome c in the intermembrane space (IMS) are transferred via the dinuclear copper A center (CU(A)) of subunit 2 and heme A of subunit 1 to the active site in subunit 1, a binuclear center (BNC) formed by heme A3 and copper B (CU(B)). The BNC reduces molecular oxygen to 2 water molecules using 4 electrons from cytochrome c in the IMS and 4 protons from the mitochondrial matrix.
Cellular Location	Mitochondrion inner membrane; Single-pass membrane protein
Tissue Location	Ubiquitous.

Background

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.

Images

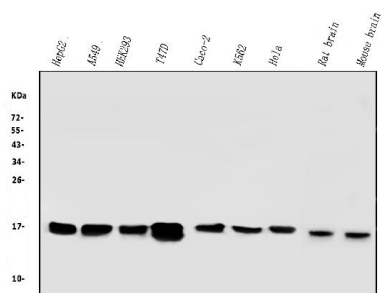


Figure 1. Western blot analysis of COX IV using anti-COX IV antibody (M05442-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions. Lane 1: human HEPG2 whole cell lysates, Lane 2: human A549 whole cell lysates, Lane 3: human HEK293 whole cell lysates, Lane 4: human T47D whole cell lysates, Lane 5: human CACO-2 whole cell lysates, Lane 6: human K562 whole cell lysates, Lane 7: human Hela whole cell lysates, Lane 8: rat brain tissue lysates, Lane 9: mouse brain 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 mouse anti-COX IV antigen affinity purified monoclonal antibody (Catalog # M05442-1) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse 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 # EK1001) with Tanon 5200 system. A specific band was detected for COX IV at approximately 17KD. The expected band size for COX IV is at 17KD.

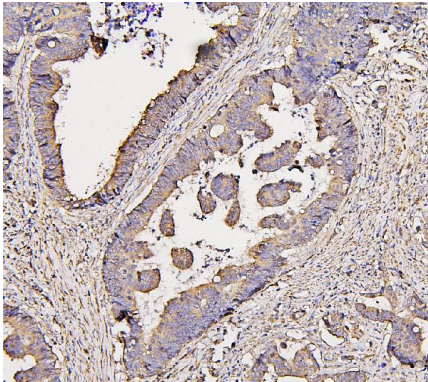


Figure 2. IHC analysis of COX IV using anti-COX IV antibody (M05442-1). COX IV was detected in paraffin-embedded section of human colon cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-COX IV Antibody (M05442-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

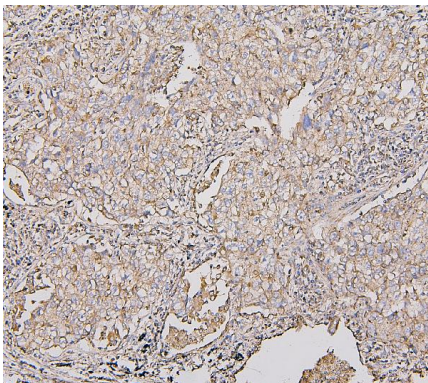


Figure 3. IHC analysis of COX IV using anti-COX IV antibody (M05442-1). COX IV was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-COX IV Antibody (M05442-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

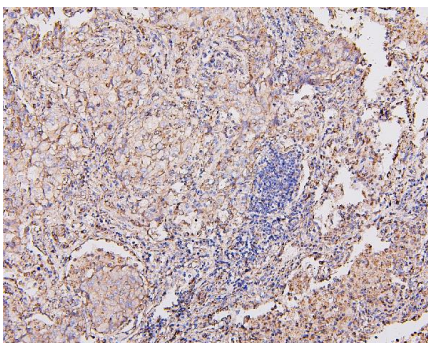
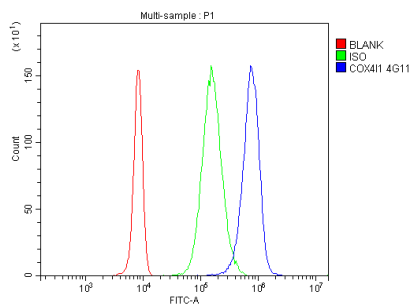


Figure 4. IHC analysis of COX IV using anti-COX IV antibody (M05442-1). COX IV was detected in paraffin-embedded section of human lung cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-COX IV Antibody (M05442-1) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

Figure 5. Flow Cytometry analysis of A431 cells using anti-COX IV antibody (M05442-1).



Overlay histogram showing A431 cells stained with M05442-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-COX IV Antibody (M05442-1, 1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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