# Anti-COX IV Antibody [JJ09-05] - BSA and Azide free HA750317



Product Type: Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human, Mouse, Rat

Applications: WB, IF-Cell, IF-Tissue, IHC-P, IP, FC

Molecular Wt: Predicted band size: 20 kDa

Clone number: JJ09-05

**Description:** Cytochrome c oxidase (COX) functions as the terminal oxidase of the respiratory chain that

uses cytochrome c as an electron donor to drive a proton gradient across the inner mitochondrial membrane. The mammalian COX apoenzyme is a heteromer consisting of three mitochondrial encoded catalytic subunits and several nuclear gene encoded structural subunits. COX contains two iron-coordination sites and two copper-coordination sites. Cytochrome c oxidase IV (COX4) is a nuclear-encoded subunit of COX that may play a role in regulating COX activity. COX4 is expressed ubiquitously in adult human tissue with the strongest levels of expression in the pancreas and moderate expression levels in heart,

skeletal muscle and placenta.

**Immunogen:** Synthetic peptide within Human COX IV aa 21-70 / 169.

Positive control: HeLa cell lysate, HepG2 cell lysate, mouse heart tissue lysate, rat heart tissue lysate,

HepG2, MCF-7, human liver tissue, human liver carcinoma tissue, human colon carcinoma

tissue, human kidney tissue, mouse colon tissue, mouse heart tissue.

**Subcellular location:** Mitochondrion inner membrane.

Database links: SwissProt: P13073 Human | P19783 Mouse | P10888 Rat

**Recommended Dilutions:** 

WB 1:1,000-1:5,000
IF-Cell 1:50-1:200
IF-Tissue 1:200

**IHC-P** 1:400-1:1,000 **FC** 1:50-1:100

**IP** Use at an assay dependent concentration.

Storage Buffer: PBS (pH7.4).

**Storage Instruction:** Store at  $+4^{\circ}$ C after thawing. Aliquot store at  $-20^{\circ}$ C or  $-80^{\circ}$ C. Avoid repeated freeze / thaw

cycles.

**Purity:** Protein A affinity purified.

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#### **Images**

 Fig1: Western blot analysis of COX IV on different lysates with Rabbit anti-COX IV antibody (HA750317) at 1/1,000 dilution.

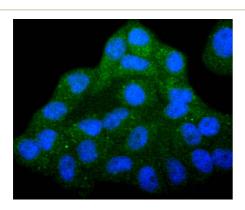
Lane 1: HeLa cell lysate (20 µg/Lane) Lane 2: HepG2 cell lysate (20 µg/Lane)

Lane 3: Mouse heart tissue lysate (30 µg/Lane) Lane 4: Rat heart tissue lysate (30 µg/Lane)

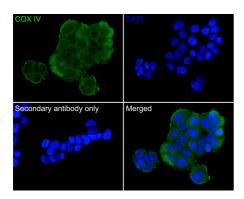
Predicted band size: 20 kDa Observed band size: 15 kDa

Exposure time: 10 seconds; ECL: K1801;

4-20% SDS-PAGE gel.



**Fig2:** ICC staining of COX IV in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750317, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

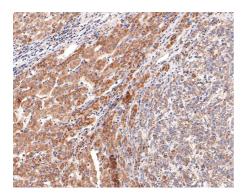


**Fig3:** Immunocytochemistry analysis of MCF-7 cells labeling COX IV with Rabbit anti-COX IV antibody (HA750317) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37  $^{\circ}$ C, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-COX IV antibody (HA750317) at 1/50 dilution in 2% negative goat serum overnight at 4  $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor \*\* 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

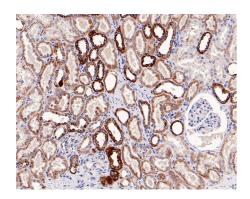
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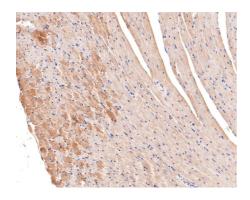
**Fig4:** Immunohistochemical analysis of paraffin-embedded human liver carcinoma tissue with Rabbit anti-COX IV antibody (HA750317) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750317) at 1/400 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig5:** Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-COX IV antibody (HA750317) at 1/800 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750317) at 1/800 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig6:** Immunohistochemical analysis of paraffin-embedded mouse heart tissue with Rabbit anti-COX IV antibody (HA750317) at 1/400 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750317) at 1/400 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

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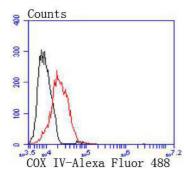
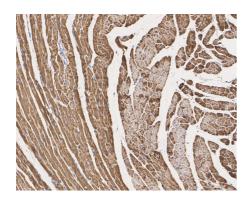


Fig7: Flow cytometric analysis of COX IV was done on MCF-7 cells. The cells were fixed, permeabilized and stained with the primary antibody (HA750317, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).



**Fig8:** Immunohistochemical analysis of paraffin-embedded rat heart tissue with Rabbit anti-COX IV antibody (HA750317) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA750317) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

### **Background References**

- 1. Sakakibara I et al. Six1 homeoprotein drives myofiber type IIA specialization in soleus muscle. Skelet Muscle 6:30 (2016).
- Cheng CY et al. Electroacupuncture at different frequencies (5Hz and 25Hz) ameliorates cerebral ischemiareperfusion injury in rats: possible involvement of p38 MAPK-mediated anti-apoptotic signaling pathways. BMC Complement Altern Med 15:241 (2015).