Anti-NDUFA8 Antibody [PSH21-96]

HA724265

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Green monkey
Applications:	WB, IHC-P, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 20 kDa
Clone number:	PSH21-96

Description: NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8 is an enzyme that in humans is encoded by the NDUFA8 gene. The NDUFA8 protein is a subunit of NADH dehydrogenase (ubiquinone), which is located in the mitochondrial inner membrane and is the largest of the five complexes of the electron transport chain. The human NDUFA8 gene codes for a subunit of Complex I of the respiratory chain, which transfers electrons from NADH to ubiquinone. NADH binds to Complex I and transfers two electrons to the isoalloxazine ring of the flavin mononucleotide (FMN) prosthetic arm to form FMNH2. The electrons are transferred through a series of iron-sulfur (Fe-S) clusters in the prosthetic arm and finally to coenzyme Q10 (CoQ), which is reduced to ubiquinol (CoQH2). The flow of electrons changes the redox state of the protein, resulting in a conformational change and pK shift of the ionizable side chain, which pumps four hydrogen ions out of the mitochondrial matrix.

Immunogen: Recombinant protein within human NDUFA8 aa 1-172.

Positive control: MCF7 cell lysate, HepG2 cell lysate, HeLa cell lysate, SiHa cell lysate, LO2 cell lysate, COS-1 cell lysate, L-929 cell lysate, RAW264.7 cell lysate, PC-12 cell lysate, C6 cell lysate, Mouse heart tissue lysate, Mouse brain tissue lysate, Rat heart tissue lysate, Rat brain tissue lysate, human brain tissue, human colon tissue, human heart tissue, human kidney tissue, human liver tissue, HepG2, L-929, C6.

Subcellular location: Mitochondrion inner membrane, Mitochondrion intermembrane space, Mitochondrion.

Database links: SwissProt: P51970 Human | Q9DCJ5 Mouse
Entrez Gene: 296658 Rat

Recommended Dilutions:

WB	1:5,000
IHC-P	1:50-1:200
IF-Cell	1:100
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

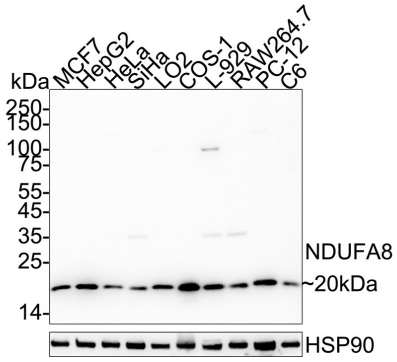

Fig1: Western blot analysis of NDUFA8 on different lysates with Rabbit anti-NDUFA8 antibody (HA724265) at 1/5,000 dilution.

Lane 1: MCF7 cell lysate (15 µg/Lane)
Lane 2: HepG2 cell lysate (15 µg/Lane)
Lane 3: HeLa cell lysate (15 µg/Lane)
Lane 4: SiHa cell lysate (15 µg/Lane)
Lane 5: LO2 cell lysate (15 µg/Lane)
Lane 6: COS-1 cell lysate (15 µg/Lane)
Lane 7: L-929 cell lysate (15 µg/Lane)
Lane 8: RAW264.7 cell lysate (15 µg/Lane)
Lane 9: PC-12 cell lysate (15 µg/Lane)
Lane 10: C6 cell lysate (15 µg/Lane)

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

Exposure time: 15 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA724265) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

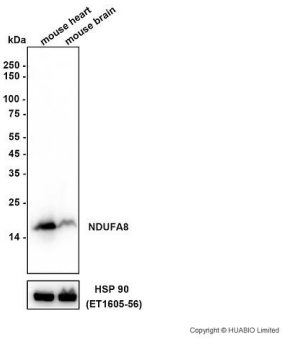

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Fig2: Western blot analysis of NDUFA8 on different lysates with Rabbit anti-NDUFA8 antibody (HA724265) at 1/5,000 dilution.

Lane 1: Mouse heart tissue lysate (30 µg/Lane)
Lane 2: Mouse brain tissue lysate (30 µg/Lane)

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

Exposure time: 2 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA724265) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

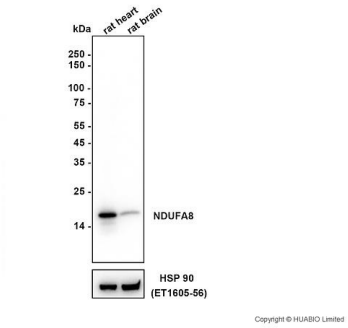

Fig3: Western blot analysis of NDUFA8 on different lysates with Rabbit anti-NDUFA8 antibody (HA724265) at 1/5,000 dilution.

Lane 1: Rat heart tissue lysate (30 µg/Lane)
Lane 2: Rat brain tissue lysate (30 µg/Lane)

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

Exposure time: 2 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA724265) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

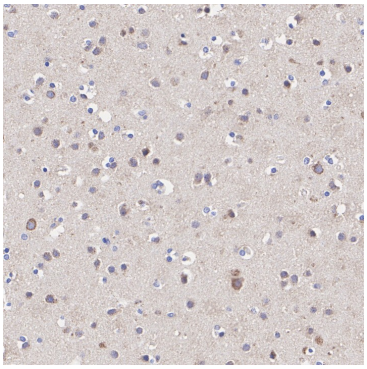

Fig4: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-NDUFA8 antibody (HA724265) at 1/50 dilution.

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_2O and PBS, and then probed with the primary antibody (HA724265) at 1/50 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.

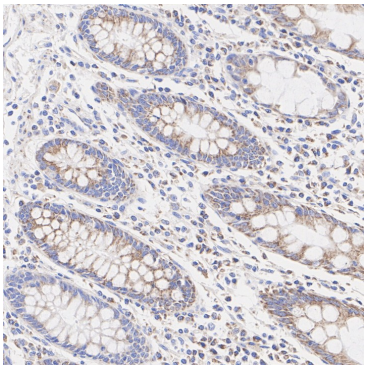

Fig5: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-NDUFA8 antibody (HA724265) at 1/50 dilution.

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_2O and PBS, and then probed with the primary antibody (HA724265) at 1/50 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.

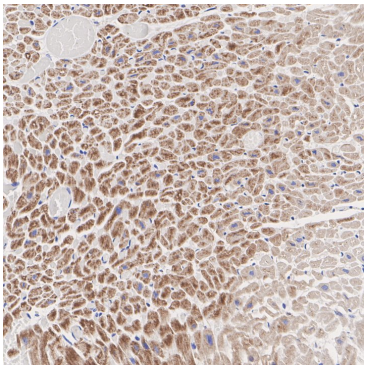

Fig6: Immunohistochemical analysis of paraffin-embedded human heart tissue with Rabbit anti-NDUFA8 antibody (HA724265) at 1/50 dilution.

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_2O and PBS, and then probed with the primary antibody (HA724265) at 1/50 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.

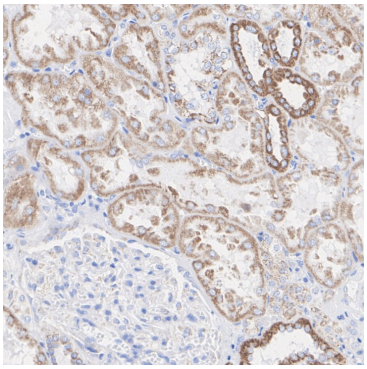

Fig7: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-NDUFA8 antibody (HA724265) at 1/200 dilution.

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_2O and PBS, and then probed with the primary antibody (HA724265) at 1/200 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.

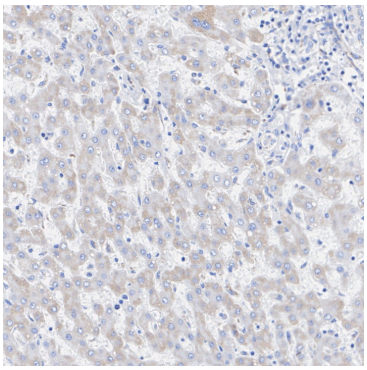

Fig8: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-NDUFA8 antibody (HA724265) at 1/200 dilution.

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_2O and PBS, and then probed with the primary antibody (HA724265) at 1/200 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.

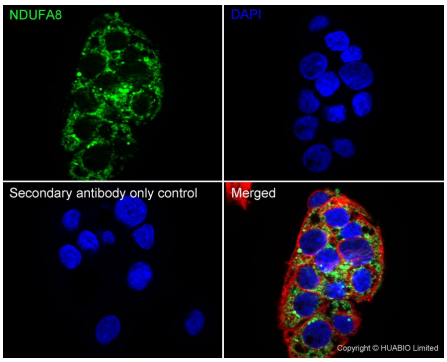

Fig9: Immunocytochemistry analysis of HepG2 cells labeling NDUFA8 with Rabbit anti-NDUFA8 antibody (HA724265) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-NDUFA8 antibody (HA724265) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

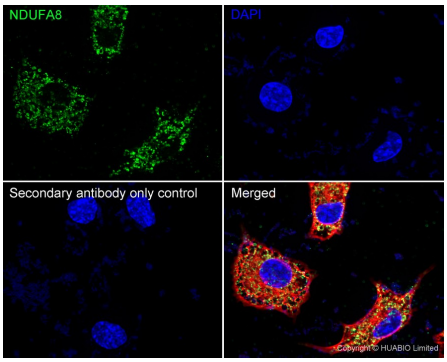

Fig10: Immunocytochemistry analysis of L-929 cells labeling NDUFA8 with Rabbit anti-NDUFA8 antibody (HA724265) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-NDUFA8 antibody (HA724265) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

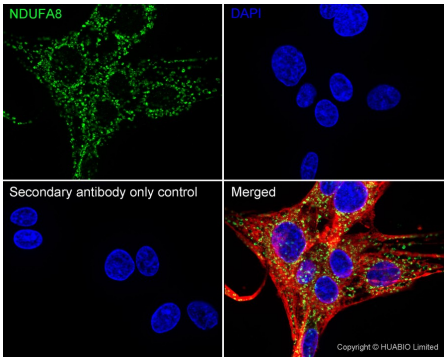

Fig11: Immunocytochemistry analysis of C6 cells labeling NDUFA8 with Rabbit anti-NDUFA8 antibody (HA724265) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-NDUFA8 antibody (HA724265) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

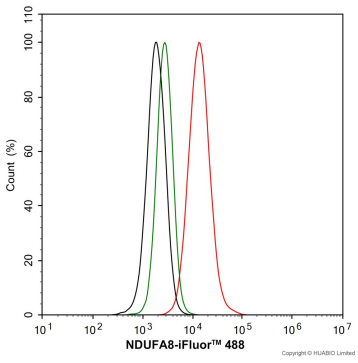

Fig12: Flow cytometric analysis of HepG2 cells labeling NDUFA8.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA724265, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

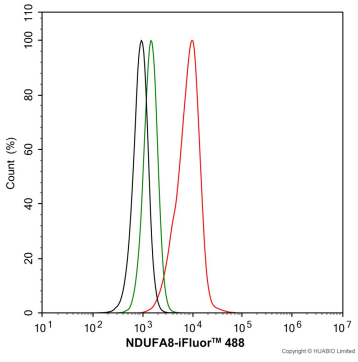

Fig13: Flow cytometric analysis of L-929 cells labeling NDUFA8.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA724265, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

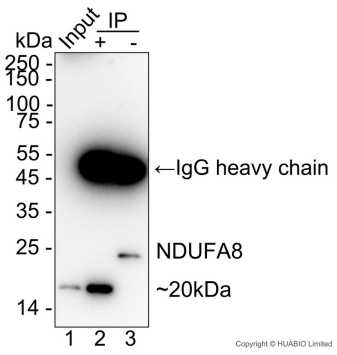

Fig14: NDUFA8 was immunoprecipitated from 0.2 mg HepG2 cell lysate with HA724265 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA724265 at 1/5,000 dilution. Alpaca anti-Rabbit IgG Fc secondary antibody (HA1031) at 1/50,000 dilution was used for 1 hour at room temperature.

Lane 1: HepG2 cell lysate (input)
Lane 2: HA724265 IP in HepG2 cell lysate
Lane 3: Rabbit IgG instead of HA724265 in HepG2 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 46 seconds; ECL: K1801

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

Background References

1. Chen J et al. NDUFA8 potentially rescues Wolbachia-induced cytoplasmic incompatibility in Laodelphax striatellus. Insect Sci. 2023 Dec
2. Xiang H et al. NDUFA8 is transcriptionally regulated by EP300/H3K27ac and promotes mitochondrial respiration to support proliferation and inhibit apoptosis in cervical cancer. Biochem Biophys Res Commun. 2024 Jan