Anti-NDUFS2 Antibody [PSH03-26] - BSA and Azide free

HA750866

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 53 kDa
Clone number:	PSH03-26

Description: Mitochondrial complex I is the first multimeric complex of the respiratory chain that catalyzes the NADH oxidation with concomitant ubiquinone reduction and proton ejection out of the mitochondria. Mammalian mitochondrial complex I is an assembly of at least 43 different subunits. Seven of the subunits are encoded by the mitochondrial genome; the remainder are the products of nuclear genes. The iron-sulfur protein (IP) fraction of complex I is made up of 7 subunits, including NDUFS2. Dimethylation at Arg-118 by NDUFAF7 takes place after NDUFS2 assembles into the complex I, leading to the stabilization of the early intermediate complex.

Immunogen: Recombinant protein within human NDUFS2 aa 1-463 / 463.

Positive control: HeLa cell lysate, Jurkat cell lysate, 293T cell lysate, A431 cell lysate, Raji cell lysate, SK-Br-3 cell lysate, K-562 cell lysate, NIH/3T3 cell lysate, RAW264.7 cell lysate, PC-12 cell lysate, mouse brain tissue lysate, mouse kidney tissue lysate, rat brain tissue lysate, rat kidney tissue lysate, human kidney tissue, human liver tissue, mouse heart tissue, mouse kidney tissue, rat kidney tissue, A431, HeLa, NIH/3T3.

Subcellular location: Mitochondrion inner membrane.

Database links: SwissProt: O75306 Human | Q91WD5 Mouse | Q641Y2 Rat

Recommended Dilutions:

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

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

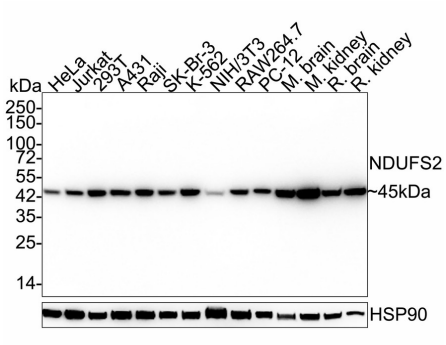

Fig1: Western blot analysis of NDUFS2 on different lysates with Rabbit anti-NDUFS2 antibody (HA750866) at 1/2,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: Jurkat cell lysate (20 µg/Lane)
Lane 3: 293T cell lysate (20 µg/Lane)
Lane 4: A431 cell lysate (20 µg/Lane)
Lane 5: Raji cell lysate (20 µg/Lane)
Lane 6: SK-Br-3 cell lysate (20 µg/Lane)
Lane 7: K-562 cell lysate (20 µg/Lane)
Lane 8: NIH/3T3 cell lysate (20 µg/Lane)
Lane 9: RAW264.7 cell lysate (20 µg/Lane)
Lane 10: PC-12 cell lysate (20 µg/Lane)
Lane 11: Mouse brain tissue lysate (40 µg/Lane)
Lane 12: Mouse kidney tissue lysate (40 µg/Lane)
Lane 13: Rat brain tissue lysate (40 µg/Lane)
Lane 14: Rat kidney tissue lysate (40 µg/Lane)

Predicted band size: 53 kDa
Observed band size: 45 kDa

Exposure time: 24 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 (HA750866) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

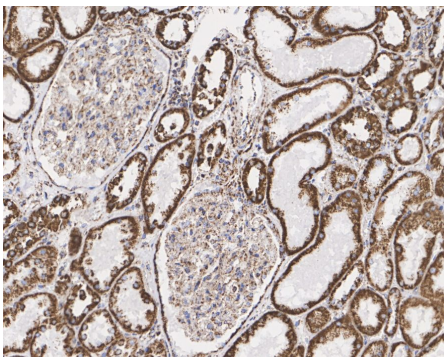

Fig2: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-NDUFS2 antibody (HA750866) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA750866) 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.

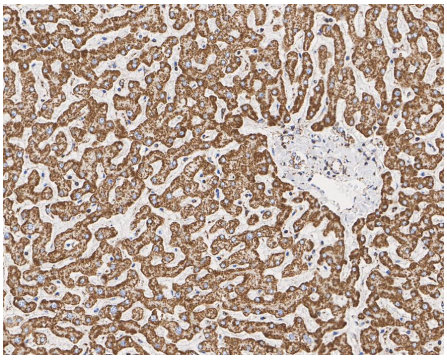

Fig3: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-NDUFS2 antibody (HA750866) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA750866) 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.

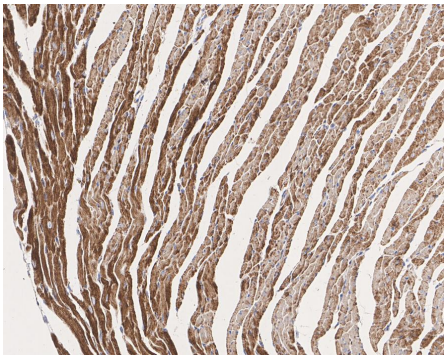

Fig4: Immunohistochemical analysis of paraffin-embedded mouse heart tissue with Rabbit anti-NDUFS2 antibody (HA750866) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA750866) 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.

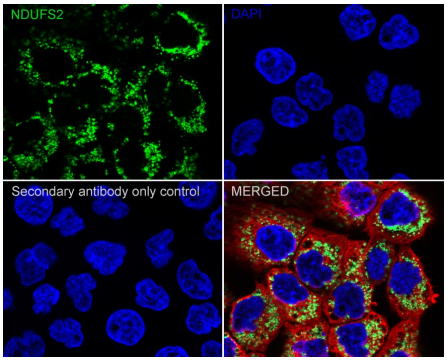

Fig5: Immunocytochemistry analysis of A431 cells labeling NDUFS2 with Rabbit anti-NDUFS2 antibody (HA750866) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-NDUFS2 antibody (HA750866) 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 (M1305-2, 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.

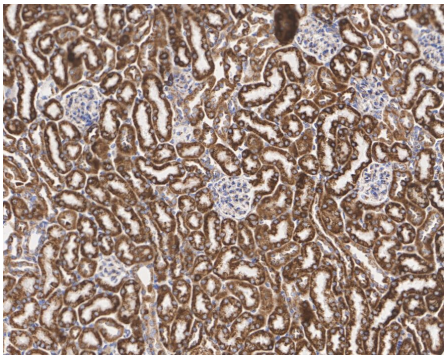

Fig6: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-NDUFS2 antibody (HA750866) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA750866) 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.

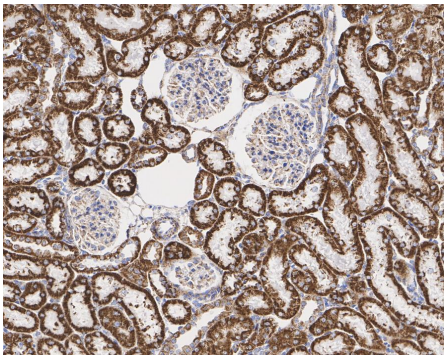

Fig7: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-NDUFS2 antibody (HA750866) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA750866) 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.

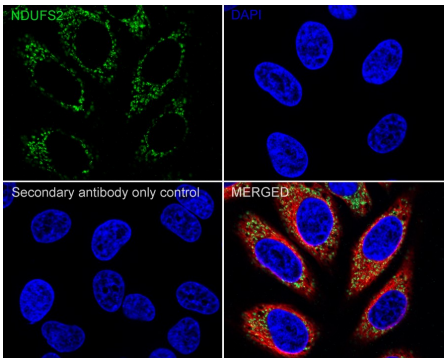

Fig8: Immunocytochemistry analysis of HeLa cells labeling NDUFS2 with Rabbit anti-NDUFS2 antibody (HA750866) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-NDUFS2 antibody (HA750866) 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 (M1305-2, 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.

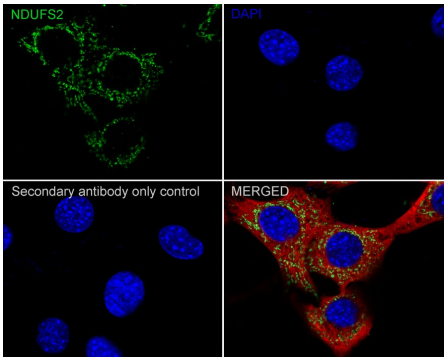

Fig9: Immunocytochemistry analysis of NIH/3T3 cells labeling NDUFS2 with Rabbit anti-NDUFS2 antibody (HA750866) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-NDUFS2 antibody (HA750866) 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 (M1305-2, 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.

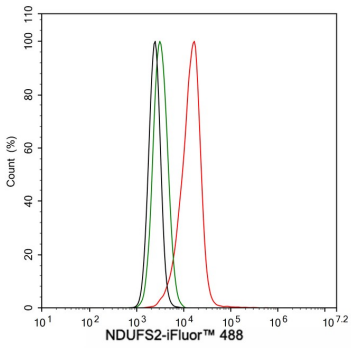

Fig10: Flow cytometric analysis of A431 cells labeling NDUFS2.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750866, 1μg/mL) (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).

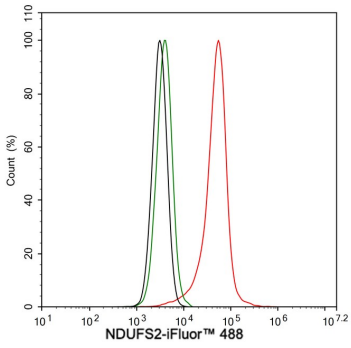

Fig11: Flow cytometric analysis of NIH/3T3 cells labeling NDUFS2.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750866, 1μg/mL) (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).

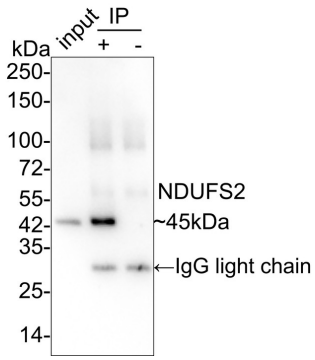

Fig12: NDUFS2 was immunoprecipitated in 0.2mg HeLa cell lysate with HA750866 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA750866 at 1/2,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: HA750866 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of HA750866 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 43 seconds

Background References

1. Bandara AB et al. Complex I protein NDUFS2 is vital for growth, ROS generation, membrane integrity, apoptosis, and mitochondrial energetics. Mitochondrion. 2021 May
2. Dunham-Snary KJ et al. Ndufs2, a Core Subunit of Mitochondrial Complex I, Is Essential for Acute Oxygen-Sensing and Hypoxic Pulmonary Vasoconstriction. Circ Res. 2019 Jun