Anti-NDUFB8 Antibody [JG61-36]

ET7108-25

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IP, IHC-P, IF-Cell
Molecular Wt:	Predicted band size: 22 kDa
Clone number:	JG61-36

Description: Accessory subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I), that is believed not to be involved in catalysis. Complex I functions in the transfer of electrons from NADH to the respiratory chain. The immediate electron acceptor for the enzyme is believed to be ubiquinone.

Immunogen: Recombinant protein within Human NDUFB8 aa 1-186 / 186.

Positive control: HeLa cell lysate, A549 cell lysate, RAW264.7 cell lysate, C6 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, HeLa, C6, human kidney tissue, mouse kidney tissue, rat kidney tissue.

Subcellular location: Mitochondrion.

Database links: SwissProt: O95169 Human | Q9D6J5 Mouse
Entrez Gene: 293991 Rat

Recommended Dilutions:

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

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

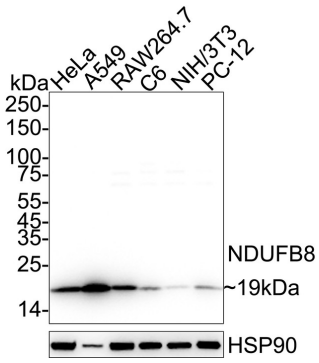

Fig1: Western blot analysis of NDUFB8 on different lysates with Rabbit anti-NDUFB8 antibody (ET7108-25) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: A549 cell lysate
Lane 3: RAW264.7 cell lysate
Lane 4: C6 cell lysate
Lane 5: NIH/3T3 cell lysate
Lane 6: PC-12 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 22 kDa
Observed band size: 19 kDa

Exposure time: 10 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 (ET7108-25) 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.

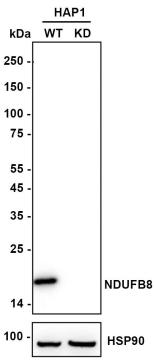

Fig2: Western blot analysis of NDUFB8 on different lysates with Rabbit anti-NDUFB8 antibody (ET7108-25) at 1/5,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-NDUFB8 KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 22 kDa
Observed band size: 19 kDa

Exposure time: 20 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 (ET7108-25) at 1/5,000 dilution was used in 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.

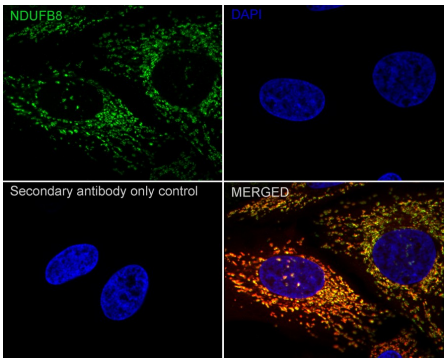

Fig3: Immunocytochemistry analysis of HeLa cells labeling NDUFB8 with Rabbit anti-NDUFB8 antibody (ET7108-25) at 1/100 dilution.

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-NDUFB8 antibody (ET7108-25) 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. Counterstained with Mitotracker. Nuclear DNA was labelled in blue with DAPI.

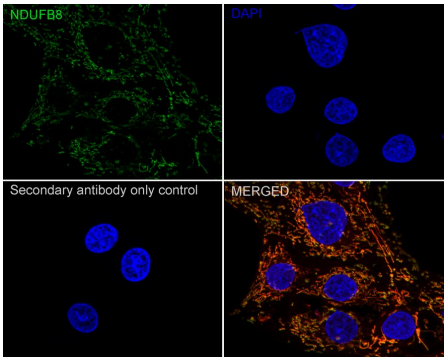

Fig4: Immunocytochemistry analysis of C6 cells labeling NDUFB8 with Rabbit anti-NDUFB8 antibody (ET7108-25) at 1/50 dilution.

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-NDUFB8 antibody (ET7108-25) at 1/50 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. Counterstained with Mitotracker. Nuclear DNA was labelled in blue with DAPI.

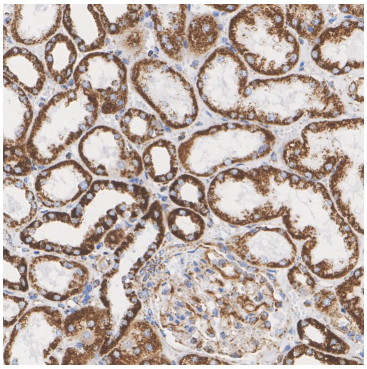

Fig5: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-NDUFB8 antibody (ET7108-25) at 1/200 dilution.

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 (ET7108-25) 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.

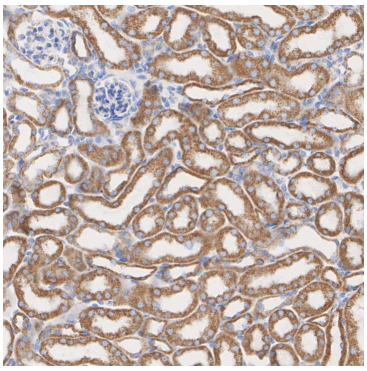

Fig6: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-NDUFB8 antibody (ET7108-25) at 1/200 dilution.

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 (ET7108-25) 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.

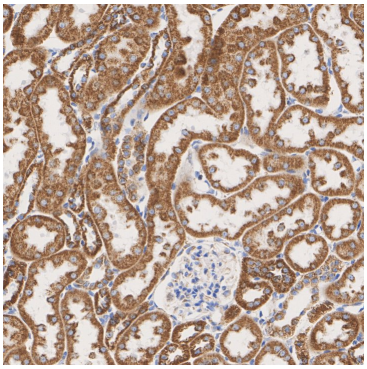

Fig7: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-NDUFB8 antibody (ET7108-25) at 1/200 dilution.

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 (ET7108-25) 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.

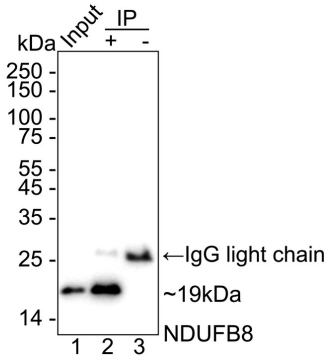

Fig8: NDUFB8 was immunoprecipitated from 0.2 mg HeLa cell lysate with ET7108-25 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET7108-25 at 1/2,000 dilution. Mouse Anti-Rabbit IgG kappa light chain secondary antibody (M1208-2) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: ET7108-25 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of ET7108-25 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 10 seconds; ECL: K1801

Background References

1. Stroud D A et al. Accessory subunits are integral for assembly and function of human mitochondrial complex I. Nature 538:123-126 (2016).
2. Murray J et al. The subunit composition of the human NADH dehydrogenase obtained by rapid one-step immunopurification. J Biol Chem 278:13619-13622 (2003).

Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation