Anti-Mitofilin Antibody [PSH01-19]

HA721636

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, IF-Tissue
Molecular Wt:	Predicted band size: 84 kDa
Clone number:	PSH01-19

Description: Mitochondrial inner membrane protein is a protein that in humans is encoded by the IMMT gene. IMMT encodes an inner mitochondrial membrane (IMM) protein in the nucleus. It is posttranslational transported to the IMM. Mic60/Mitofilin (encoded by the IMMT gene) is a core subunit of the MICOS-complex, directly located next to cristae junctions (CJ). Human Mic60 exists in two isoforms of different size, anchored to the IMM via its N-terminus, while most of the protein is located to the inner mitochondrial space (IMS). Mic60 is evolutionary one of the oldest MICOS subunits as homologous were found in anaerobic prokaryotes. It is mainly present in two isoforms (ca. 88 and 90 kDa). In the brain, four isoforms are known, which differ in their isoelectric point due to different post-translational modifications. The amino terminus of Mic60 is anchored in the IM, while most of the protein is extended to the IMS. C-terminal Mic60 has a conserved mitofilin domain which is crucial for building the MICOS-complex. A central coiled-coil domain is required to enable protein-protein interactions.

Immunogen: Recombinant protein within human Mitofilin aa 151-400 / 758.

Positive control: HeLa cell lysate, Raji cell lysate, HEK-293 cell lysate, HepG2 cell lysate, MCF7 cell lysate, Saos-2 cell lysate, human liver carcinoma tissue, human pancreas tissue, human thyroid carcinoma tissue, mouse brain tissue, rat brain tissue, HeLa.

Subcellular location: Mitochondrion inner membrane, Mitochondrion.

Database links: SwissProt: Q16891 Human | Q8CAQ8 Mouse | Q3KR86 Rat

Recommended Dilutions:

WB	1:100,000
IHC-P	1:1,000
IF-Cell	1:250
IF-Tissue	1:200

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

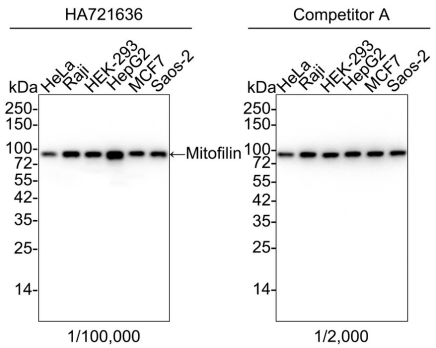

Fig1: Western blot analysis of Mitofilin on different lysates with Rabbit anti-Mitofilin antibody (HA721636) at 1/100,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: Raji cell lysate
Lane 3: HEK-293 cell lysate
Lane 4: HepG2 cell lysate
Lane 5: MCF7 cell lysate
Lane 6: Saos-2 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 84 kDa
Observed band size: 84 kDa

Exposure time: 21 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 (HA721636) at 1/100,000 dilution and competitor's antibody at 1/2,000 dilution were 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.

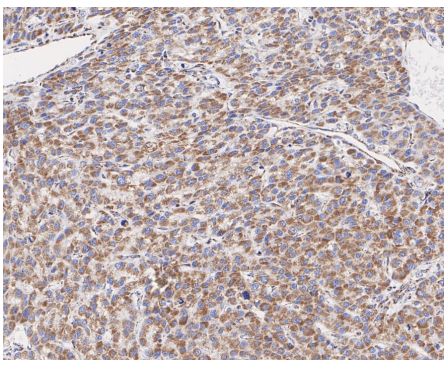

Fig2: Immunohistochemical analysis of paraffin-embedded human liver carcinoma tissue with Rabbit anti-Mitofilin antibody (HA721636) at 1/1,000 dilution.

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 (HA721636) 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.

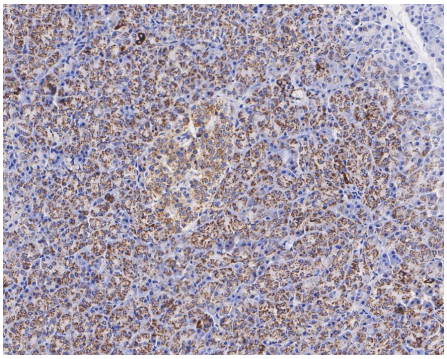

Fig3: Immunohistochemical analysis of paraffin-embedded human pancreas tissue with Rabbit anti-Mitofilin antibody (HA721636) at 1/1,000 dilution.

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 (HA721636) 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.

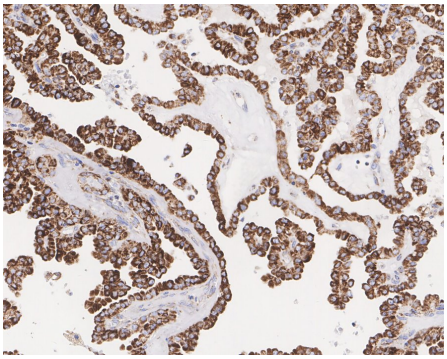

Fig4: Immunohistochemical analysis of paraffin-embedded human thyroid carcinoma tissue with Rabbit anti-Mitofilin antibody (HA721636) at 1/1,000 dilution.

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 (HA721636) 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.

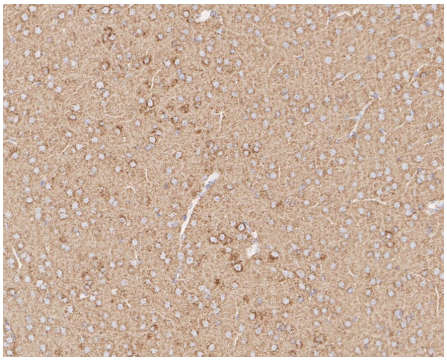

Fig5: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-Mitofilin antibody (HA721636) at 1/5,000 dilution.

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 (HA721636) at 1/5,000 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.

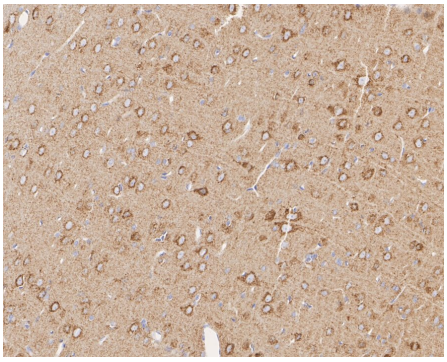

Fig6: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Mitofilin antibody (HA721636) at 1/5,000 dilution.

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 (HA721636) at 1/5,000 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.

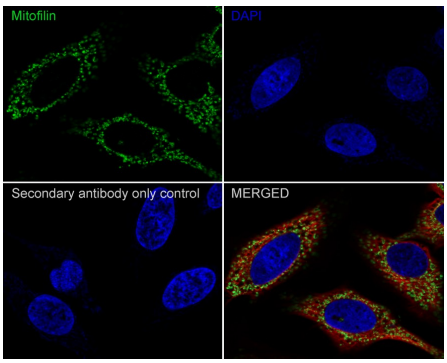

Fig7: Immunocytochemistry analysis of HeLa cells labeling Mitofilin with Rabbit anti-Mitofilin antibody (HA721636) at 1/250 dilution.

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-Mitofilin antibody (HA721636) at 1/250 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.

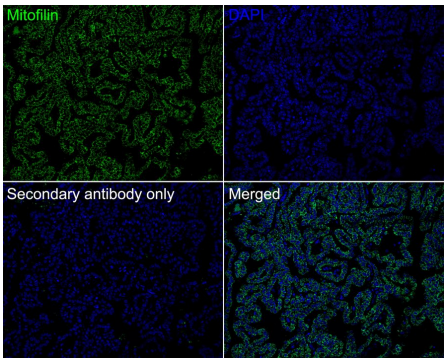

Fig8: Immunofluorescence analysis of paraffin-embedded human thyroid carcinoma tissue labeling Mitofilin with Rabbit anti-Mitofilin antibody (HA721636) at 1/200 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721636, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

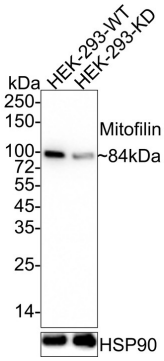

Fig9: Western blot analysis of Mitofilin on different lysates with Rabbit anti-Mitofilin antibody (HA721636) at 1/10,000 dilution.

Lane 1: HEK-293-si NT cell lysate
Lane 2: HEK-293-si Mitofilin cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 84 kDa
Observed band size: 84 kDa

Exposure time: 5 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 (HA721636) at 1/10,000 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.

Background References

1. Ma M et al. Mitofilin Mitigates Myocardial Damage in Acute Myocardial Infarction by Regulating Pyroptosis of Cardiomyocytes. Front Cardiovasc Med. 2022 May
2. Feng Y et al. RIP3 Translocation into Mitochondria Promotes Mitofilin Degradation to Increase Inflammation and Kidney Injury after Renal Ischemia-Reperfusion. Cells. 2022 Jun