Anti-HADHA Antibody [PSH01-28]

HA721652

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IHC-P, IF-Cell
Molecular Wt:	Predicted band size: 83 kDa
Clone number:	PSH01-28

Description: Trifunctional enzyme subunit alpha, mitochondrial also known as hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), alpha subunit is a protein that in humans is encoded by the HADHA gene. Mutations in HADHA have been associated with trifunctional protein deficiency or long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. This gene encodes the alpha subunit of the mitochondrial trifunctional protein, which catalyzes the last three steps of mitochondrial beta-oxidation of long chain fatty acids. The enzyme converts medium- and long-chain 2-enoyl-CoA compounds into the following 3-ketoacyl-CoA when NAD is solely present, and acetyl-CoA when NAD and CoASH are present. The alpha subunit catalyzes this reaction, and is attached to HADHB, which catalyzes the last step of the reaction.

Immunogen: Recombinant protein within human HADHA aa 1-763 / 763.

Positive control: 293T cell lysate, HeLa cell lysate, HepG2 cell lysate, Jurkat cell lysate, SH-SY5Y cell lysate, COS-1 cell lysate, mouse heart tissue lysate, mouse kidney tissue lysate, human kidney tissue lysate, rat liver tissue lysate, HeLa, human heart tissue, human kidney tissue, mouse heart tissue, mouse kidney tissue, rat heart tissue.

Subcellular location: Mitochondrion, Mitochondrion inner membrane.

Database links: SwissProt: P40939 Human | Q8BMS1 Mouse | Q64428 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:1,000
IF-Cell	1:100

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

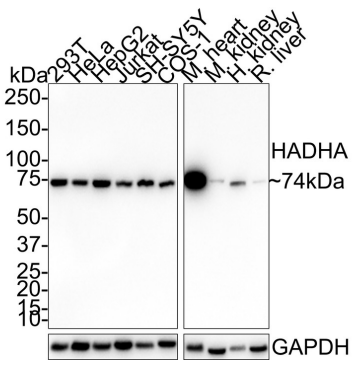

Fig1: Western blot analysis of HADHA on different lysates with Rabbit anti-HADHA antibody (HA721652) at 1/1,000 dilution.

Lane 1: 293T cell lysate
Lane 2: HeLa cell lysate
Lane 3: HepG2 cell lysate
Lane 4: Jurkat cell lysate
Lane 5: SH-SY5Y cell lysate
Lane 6: COS-1 cell lysate
Lane 7: Mouse heart tissue lysate
Lane 8: Mouse kidney tissue lysate
Lane 9: Human kidney tissue lysate
Lane 10: Rat liver tissue lysate

Lysates/proteins at 30 µg/Lane.

Predicted band size: 83 kDa
Observed band size: 74 kDa

Exposure time: 1 minute 40 seconds;
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 (HA721652) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

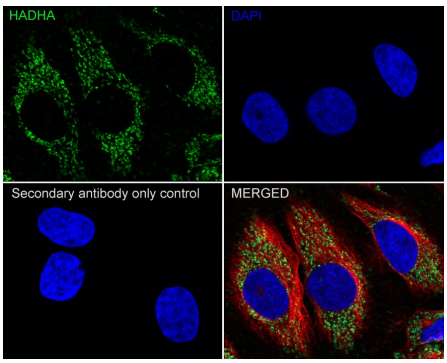

Fig2: Immunocytochemistry analysis of HeLa cells labeling HADHA with Rabbit anti-HADHA antibody (HA721652) at 1/100 dilution.

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-HADHA antibody (HA721652) 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.

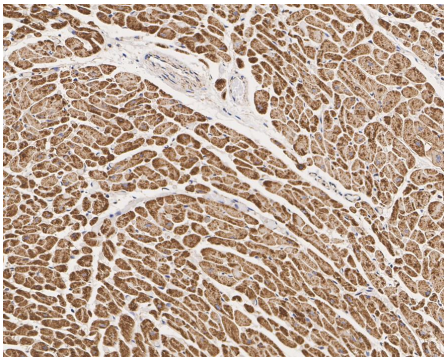

Fig3: Immunohistochemical analysis of paraffin-embedded human heart tissue with Rabbit anti-HADHA antibody (HA721652) at 1/1,000 dilution.

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 (HA721652) 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.

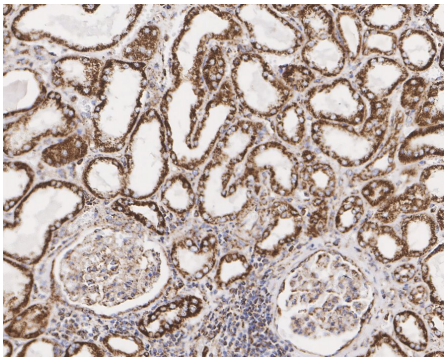

Fig4: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-HADHA antibody (HA721652) at 1/1,000 dilution.

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 (HA721652) 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.

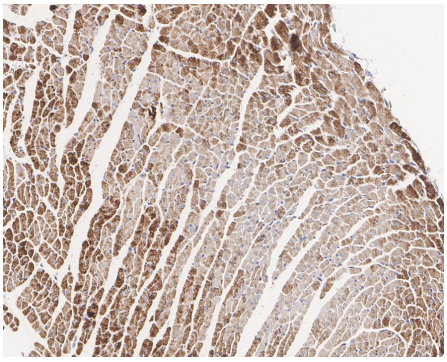

Fig5: Immunohistochemical analysis of paraffin-embedded mouse heart tissue with Rabbit anti-HADHA antibody (HA721652) at 1/1,000 dilution.

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 (HA721652) 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.

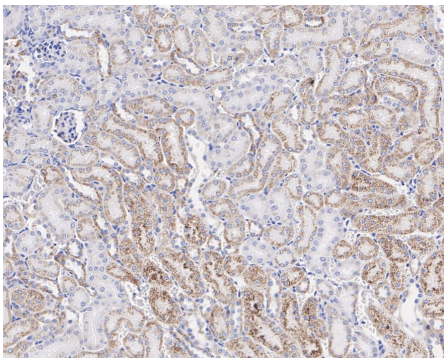

Fig6: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-HADHA antibody (HA721652) at 1/1,000 dilution.

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 (HA721652) 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.

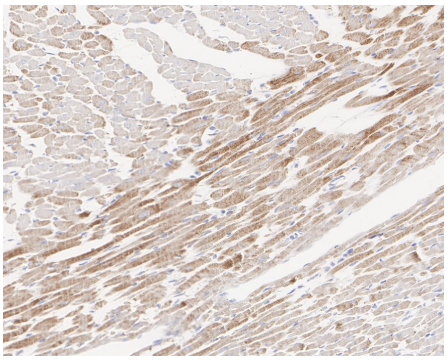

Fig7: Immunohistochemical analysis of paraffin-embedded rat heart tissue with Rabbit anti-HADHA antibody (HA721652) at 1/1,000 dilution.

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 (HA721652) 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.

Background References

1. Pan A et al. The mitochondrial β-oxidation enzyme HADHA restrains hepatic glucagon response by promoting β-hydroxybutyrate production. Nat Commun. 2022 Jan
2. Liu Y et al. MiR-612 regulates invadopodia of hepatocellular carcinoma by HADHA-mediated lipid reprogramming. J Hematol Oncol. 2020 Feb