Anti-Phospho-PDHA1 (S293) Antibody [JE58-27] HA721443

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, ICC, IHC-P
Molecular Wt:	Predicted band size: 43 kDa
Clone number:	JE58-27

Description: Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial is an enzyme that in humans is encoded by the PDHA1 gene.The pyruvate dehydrogenase complex is a nuclear-encoded mitochondrial matrix multienzyme complex that provides the primary link between glycolysis and the tricarboxylic acid (TCA) cycle by catalyzing the irreversible conversion of pyruvate into acetyl-CoA. The PDH complex is composed of multiple copies of 3 enzymes: E1 (PDHA1); dihydrolipoyl transacetylase (DLAT) (E2; EC 2.3.1.12); and dihydrolipoyl dehydrogenase (DLD) (E3; EC 1.8.1.4). The E1 enzyme is a heterotetramer of 2 alpha and 2 beta subunits. The E1-alpha subunit contains the E1 active site and plays a key role in the function of the PDH complex.

Immunogen: Synthetic phosphopeptide corresponding to residues surrounding Ser293 of human PDHA1 protein.

Positive control: A549 cell lysate, Jurkat cell lysate, HepG2 cell lysate, HeLa cell lysate, HEK-293 cell lysate, human kidney tissue lysate, mouse brain tissue lysate, mouse heart tissue lysate, rat kidney tissue lysate, mouse kidney tissue lysate, Jurkat, human renal clear cell carcinoma tissue, mouse brain tissue, rat colon tissue.

Subcellular location: Mitochondrion matrix.

Database links: SwissProt: P08559 Human | P35486 Mouse | P26284 Rat

Recommended Dilutions:

WB	1:1,000
ICC	1:100
IHC-P	1:500-1:2,000

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

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

Purity: Protein A affinity purified.

Images

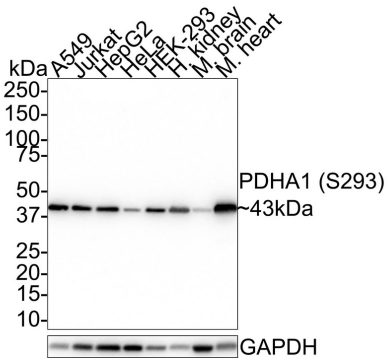

Fig1: Western blot analysis of Phospho-PDHA1 (S293) on different lysates with Rabbit anti-Phospho-PDHA1 (S293) antibody (HA721443) at 1/1,000 dilution.

Lane 1: A549 cell lysate (20 µg/Lane)
Lane 2: Jurkat cell lysate (20 µg/Lane)
Lane 3: HepG2 cell lysate (20 µg/Lane)
Lane 4: HeLa cell lysate (20 µg/Lane)
Lane 5: HEK-293 cell lysate (20 µg/Lane)
Lane 6: Human kidney tissue lysate (40 µg/Lane)
Lane 7: Mouse brain tissue lysate (40 µg/Lane)
Lane 8: Mouse heart tissue lysate (40 µg/Lane)

Predicted band size: 43 kDa
Observed band size: 43 kDa

Exposure time: 39 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 (HA721443) 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.

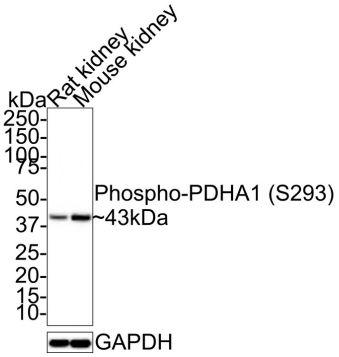

Fig2: Western blot analysis of Phospho-PDHA1 (S293) on different lysates with Rabbit anti-Phospho-PDHA1 (S293) antibody (HA721443) at 1/1,000 dilution.

Lane 1: Rat kidney tissue lysate
Lane 2: Mouse kidney tissue lysate

Lysates/proteins at 30 µg/Lane.

Predicted band size: 43 kDa
Observed band size: 43 kDa

Exposure time: 10 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 (HA721443) 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.

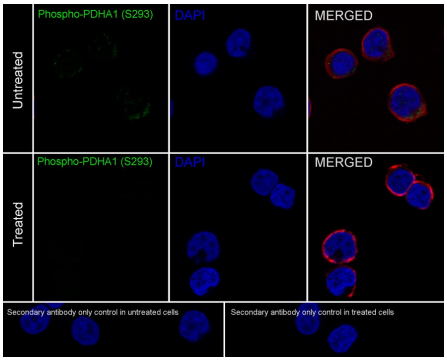

Fig3: Immunocytochemistry analysis of Jurkat cells treated with or without λpp labeling Phospho-PDHA1 (S293) with Rabbit anti-Phospho-PDHA1 (S293) antibody (HA721443) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Phospho-PDHA1 (S293) antibody (HA721443) at 1/100 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/200 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

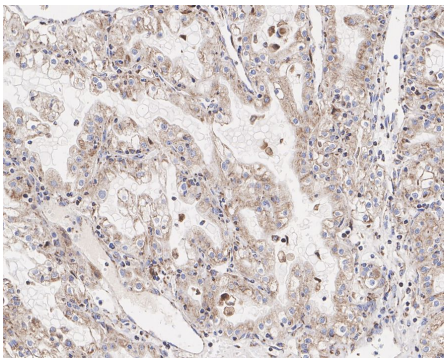

Fig4: Immunohistochemical analysis of paraffin-embedded human renal clear cell carcinoma tissue with Rabbit anti-Phospho-PDHA1 (S293) antibody (HA721443) at 1/500 dilution.

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 (HA721443) at 1/500 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.

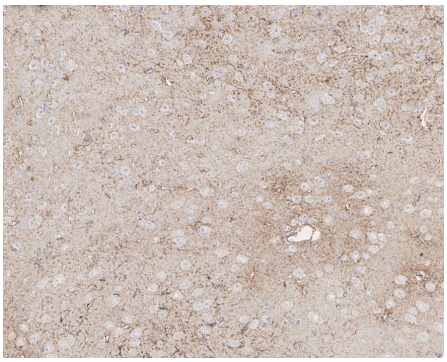

Fig5: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-Phospho-PDHA1 (S293) antibody (HA721443) at 1/500 dilution.

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 (HA721443) at 1/500 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.

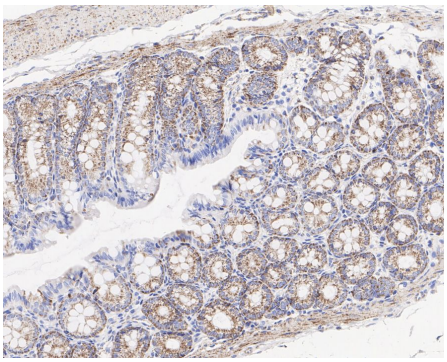

Fig6: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-Phospho-PDHA1 (S293) antibody (HA721443) at 1/2,000 dilution.

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 (HA721443) at 1/2,000 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. Deng L et al. Comprehensive analyses of PDHA1 that serves as a predictive biomarker for immunotherapy response in cancer. Front Pharmacol. 2022 Aug
2. Chen W et al. Conditional Knockout of Pdha1 in Mouse Hippocampus Impairs Cognitive Function: The Possible Involvement of Lactate. Front Neurosci. 2021 Oct