Anti-PCK2 Antibody [JB52-39]

ET7107-29

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 71 kDa
Clone number:	JB52-39

Description: As a PCK, PCK2 catalyzes the GTP-driven conversion of OAA to PEP as a rate-limiting step in gluconeogenesis. This conversion step serves as a bridge between glycolytic and TCA cycle intermediates in the mitochondria. In pancreatic β-cells, PCK2 regulates glucose-stimulated insulin secretion by recycling GTP generated from the succinyl-CoA synthase reaction. This drives the TCA cycle, converting PEP to pyruvate to acetyl-CoA for the citrate synthase reaction.[9] Since nearly all of the glycolytic reactions upstream of PEP and downstream of glucose-6-phosphate (G6P) are reversible, PCK2-mediated synthesis of PEP could fuel multiple biosynthetic processes, such as serine synthesis, glycerol synthesis, and nucleotide synthesis. Notably, PCK2 preferentially converts OAA derived from lactate and, thus, can promote biosynthesis even under low-glucose conditions. As a result, PCK2 activity contributes to cell growth and survival during stress. While PCK1 is mainly expressed in the liver and kidney, PCK2 is ubiquitously expressed in various cell types, including leukocytes and neurons, as well as in non-gluconeogenic tissues, including pancreas, brain, heart. Moreover, while PCK1 expression is regulated by hormones or nutrients involved in gluconeogenesis, PCK2 is constitutively expressed. These differences indicate that PCK2 may also perform non-gluconeogenic functions.

Immunogen:	Recombinant protein within C-terminal Human PCK2 .
Positive control:	Mouse colon tissue lysate, MCF-7 cell lysate, human kidney tissue lysate, MCF7, Neuro-2a, mouse colon tissue, human kidney tissue, human liver tissue, rat kidney tissue, human colon cancer tissue.
Subcellular location:	Mitochondrion.
Database links:	SwissProt: Q16822 Human | Q8BH04 Mouse Entrez Gene: 361042 Rat
Recommended Dilutions:	
WB	1:1,000-1:2,000
IF-Cell	1:100
IHC-P	1:50-1:200
FC	1:50-1:100
Storage Buffer:	1*TBS (pH7.4), 0.05% 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

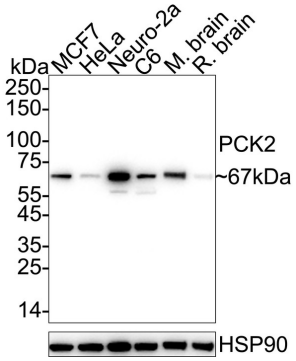

Fig1: Western blot analysis of PCK2 on different lysates with Rabbit anti-PCK2 antibody (ET7107-29) at 1/2,000 dilution.

Lane 1: MCF7 cell lysate (20 µg/Lane)
Lane 2: HeLa cell lysate (20 µg/Lane)
Lane 3: Neuro-2a cell lysate (20 µg/Lane)
Lane 4: C6 cell lysate (20 µg/Lane)
Lane 5: Mouse brain tissue lysate (40 µg/Lane)
Lane 6: Rat brain tissue lysate (40 µg/Lane)

Predicted band size: 71 kDa
Observed band size: 67 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 (ET7107-29) 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.

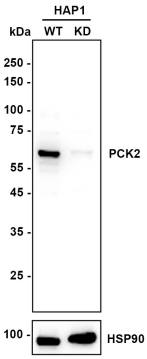

Fig2: Western blot analysis of PCK2 on different lysates with Rabbit anti-PCK2 antibody (ET7107-29) at 1/1,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-PCK2 KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 71 kDa
Observed band size: 67 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 (ET7107-29) at 1/1,000 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.

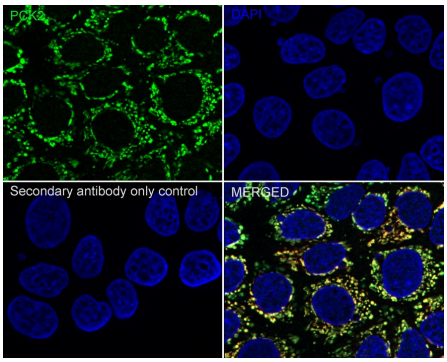

Fig3: Immunocytochemistry analysis of MCF7 cells labeling PCK2 with Rabbit anti-PCK2 antibody (ET7107-29) at 1/100 dilution.

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-PCK2 antibody (ET7107-29) 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.

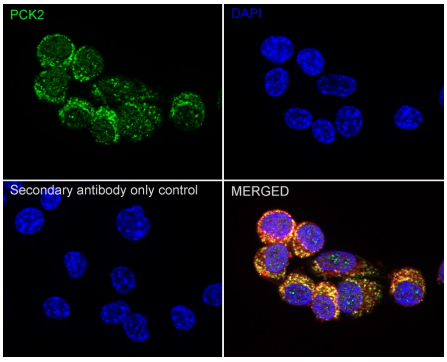

Fig4: Immunocytochemistry analysis of Neuro-2a cells labeling PCK2 with Rabbit anti-PCK2 antibody (ET7107-29) at 1/100 dilution.

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-PCK2 antibody (ET7107-29) 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.

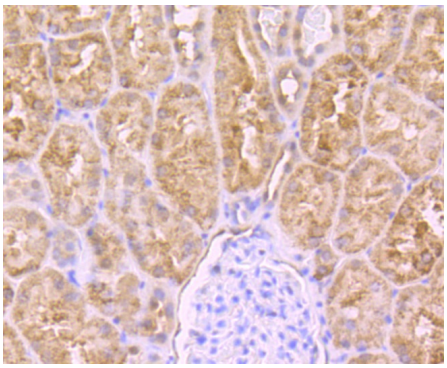

Fig5: Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-PCK2 antibody. Counter stained with hematoxylin.

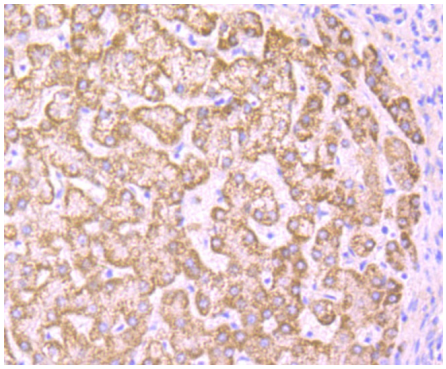

Fig6: Immunohistochemical analysis of paraffin-embedded human liver tissue using anti-PCK2 antibody. Counter stained with hematoxylin.

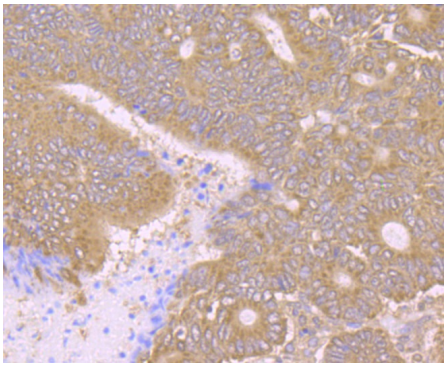

Fig7: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue using anti-PCK2 antibody. Counter stained with hematoxylin.

Background References

1. Ren Z et al. Enhancement of porcine intramuscular fat content by overexpression of the cytosolic form of phosphoenolpyruvate carboxykinase in skeletal muscle. Sci Rep 7:43746 (2017).
2. Méndez-Lucas A et al. Mitochondrial PEPCK is a Pro-Survival, ER-Stress Response Gene Involved in Tumor Cell Adaptation to Nutrient Availability. J Biol Chem 289(32):22090-102 (2014).