Anti-PCB Antibody [PSH01-55]

HA721698

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 130 kDa
Clone number:	PSH01-55

Description: Pyruvate carboxylase (PC) encoded by the gene PC is an enzyme (EC 6.4.1.1) of the ligase class that catalyzes (depending on the species) the physiologically irreversible carboxylation of pyruvate to form oxaloacetate (OAA). It is an important anaplerotic reaction that creates oxaloacetate from pyruvate. PC contains a biotin prosthetic group and is typically localized to the mitochondria in eukaryotes with exceptions to some fungal species such as Aspergillus nidulans which have a cytosolic PC. PC requires magnesium and zinc or manganese for catalysis. PC from different organisms exhibit varying degrees of activation by acetyl-CoA, but vertebrate PC typically requires it for activity.

Immunogen: Recombinant protein within Human PCB aa 450-1178.

Positive control: HepG2 cell lysate, MCF7 cell lysate, A549 cell lysate, Huh7 cell lysate, HeLa cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, Mouse kidney tissue lysate, Mouse liver tissue lysate, Mouse lymph node tissue lysate, Rat liver tissue lysate, Rat brain tissue lysate, HepG2, PC-12, mouse liver tissue, mouse lymph node tissue, rat liver tissue, rat brain tissue.

Subcellular location: Mitochondrion matrix.

Database links: SwissProt: P11498 Human | Q05920 Mouse | P52873 Rat

Recommended Dilutions:

WB	1:1,000-1:5,000
IHC-P	1:200
IF-Cell	1:250
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: PBS (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

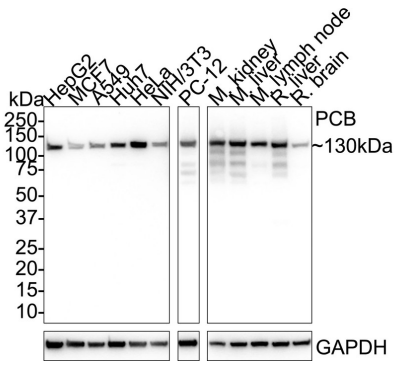

Fig1: Western blot analysis of PCB on different lysates with Rabbit anti-PCB antibody (HA721698) at 1/1,000 dilution.

Lane 1: HepG2 cell lysate (30 µg/Lane)
Lane 2: MCF7 cell lysate (30 µg/Lane)
Lane 3: A549 cell lysate (30 µg/Lane)
Lane 4: Huh7 cell lysate (30 µg/Lane)
Lane 5: HeLa cell lysate (30 µg/Lane)
Lane 6: NIH/3T3 cell lysate (30 µg/Lane)
Lane 7: PC-12 cell lysate (30 µg/Lane)
Lane 8: Mouse kidney tissue lysate (30 µg/Lane)
Lane 9: Mouse liver tissue lysate (30 µg/Lane)
Lane 10: Mouse lymph node tissue lysate (30 µg/Lane)
Lane 11: Rat liver tissue lysate (30 µg/Lane)
Lane 12: Rat brain tissue lysate (30 µg/Lane)

Predicted band size: 130 kDa
Observed band size: 130 kDa

Exposure time: Lane 1-6: 24 seconds; Lane 7-12: 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 (HA721698) 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 1/50,000 dilution was used for 1 hour at room temperature.

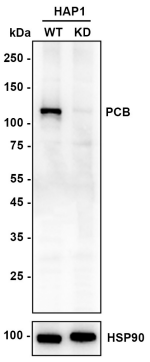

Fig2: Western blot analysis of PCB on different lysates with Rabbit anti-PCB antibody (HA721698) at 1/2,000 dilution.

Lane 1: HAP1-parental cell lysate (10 µg/Lane)
Lane 2: HAP1-PCB KD cell lysate (10 µg/Lane)

Predicted band size: 130 kDa
Observed band size: 130 kDa

Exposure time: 1 minute; 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 (HA721698) at 1/2,000 dilution was used in primary antibody dilution (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.

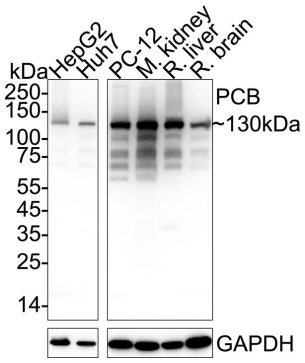

Fig3: Western blot analysis of PCB on different lysates with Rabbit anti-PCB antibody (HA721698) at 1/2,000 dilution.

Lane 1: HepG2 cell lysate (20 µg/Lane)
Lane 2: Huh7 cell lysate (20 µg/Lane)
Lane 3: PC-12 cell lysate (20 µg/Lane)
Lane 4: Mouse kidney tissue lysate (40 µg/Lane)
Lane 5: Rat liver tissue lysate (40 µg/Lane)
Lane 6: Rat brain tissue lysate (40 µg/Lane)

Predicted band size: 130 kDa
Observed band size: 130 kDa

Exposure time: 2 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 (HA721698) 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.

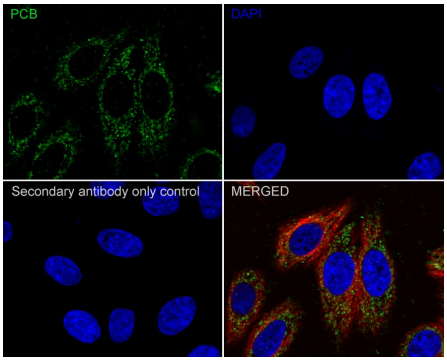

Fig4: Immunocytochemistry analysis of HepG2 cells labeling PCB with Rabbit anti-PCB antibody (HA721698) at 1/100 dilution.

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-PCB antibody (HA721698) 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.

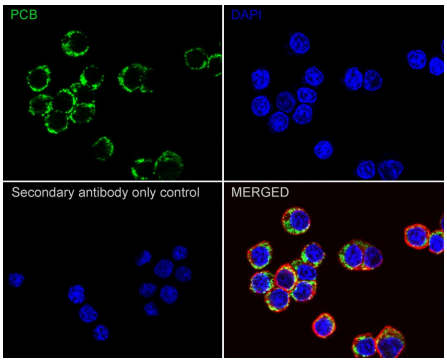

Fig5: Immunocytochemistry analysis of PC-12 cells labeling PCB with Rabbit anti-PCB antibody (HA721698) at 1/250 dilution.

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-PCB antibody (HA721698) 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.

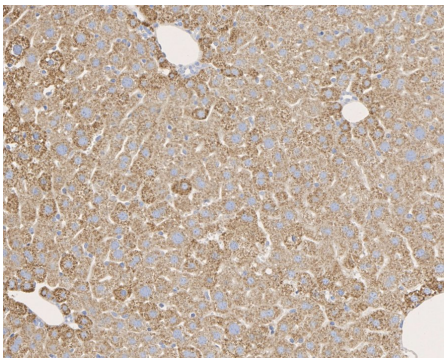

Fig6: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-PCB antibody (HA721698) at 1/200 dilution.

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 (HA721698) 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.

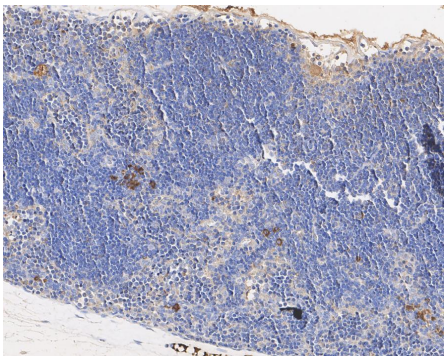

Fig7: Immunohistochemical analysis of paraffin-embedded mouse lymph node tissue with Rabbit anti-PCB antibody (HA721698) at 1/200 dilution.

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 (HA721698) 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.

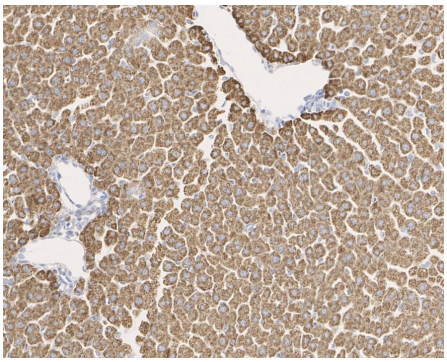

Fig8: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-PCB antibody (HA721698) at 1/200 dilution.

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 (HA721698) 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.

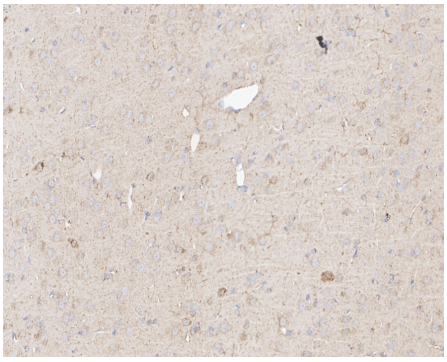

Fig9: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-PCB antibody (HA721698) at 1/200 dilution.

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 (HA721698) 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.

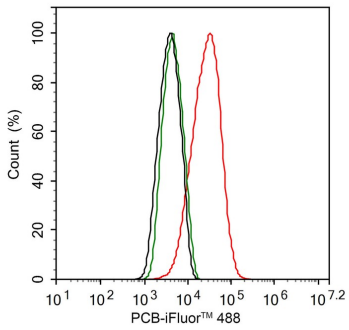

Fig10: Flow cytometric analysis of PC-12 cells labeling PCB.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721698, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

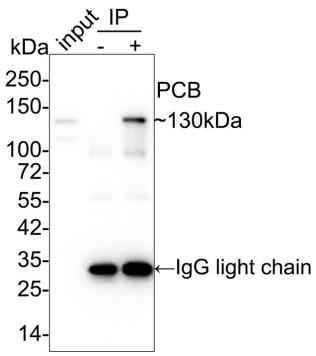

Fig11: PCB was immunoprecipitated in 0.2mg HeLa cell lysate with HA721698 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA721698 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: Rabbit IgG instead of HA721698 in HeLa cell lysate
Lane 3: HA721698 IP in HeLa cell lysate
Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 24 seconds

Background References

1. Kiesel VA et al. Pyruvate carboxylase and cancer progression. Cancer Metab. 2021 Apr
2. Schwörer S et al. Fibroblast pyruvate carboxylase is required for collagen production in the tumour microenvironment. Nat Metab. 2021 Nov