

# Anti-PCB Antibody

## ER64531



<b>Product Type:</b>	Rabbit polyclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human, Mouse, Rat
<b>Applications:</b>	WB, IF-Cell, IHC-P, FC
<b>Molecular Wt:</b>	Predicted band size: 130 kDa

**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, Huh7 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, Mouse kidney tissue lysate, Rat liver tissue lysate, Rat brain tissue lysate, HepG2, NIH/3T3, mouse liver tissue, rat liver tissue.

**Subcellular location:** Mitochondrion matrix.

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

### Recommended Dilutions:

<b>WB</b>	1:5,000-1:20,000
<b>IF-Cell</b>	1:500
<b>IHC-P</b>	1:1,000
<b>FC</b>	1:1,000

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

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

**Purity:** Immunogen affinity purified.

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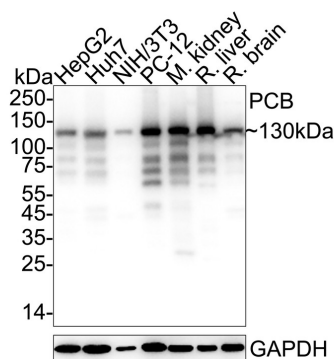
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## Images

**Fig1:** Western blot analysis of PCB on different lysates with Rabbit anti-PCB antibody (ER64531) at 1/5,000 dilution.

Lane 1: HepG2 cell lysate  
 Lane 2: Huh7 cell lysate  
 Lane 3: NIH/3T3 cell lysate  
 Lane 4: PC-12 cell lysate  
 Lane 5: Mouse kidney tissue lysate  
 Lane 6: Rat liver tissue lysate  
 Lane 7: Rat brain tissue lysate

Lysates/proteins at 20 µg/Lane.

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

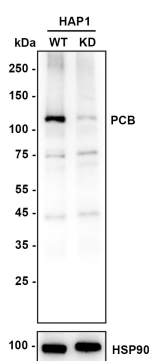
Exposure time: 2 seconds; ECL: K1801;

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 (ER64531) at 1/5,000 dilution was used in 5% NFDM/TBST at 4°C 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 PCB on different lysates with Rabbit anti-PCB antibody (ER64531) at 1/10,000 dilution.

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



Lysates/proteins at 10 µg/Lane.

Predicted band size: 130 kDa  
 Observed band size: 130 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 (ER64531) at 1/10,000 dilution was used in primary antibody dilution (K1803) at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

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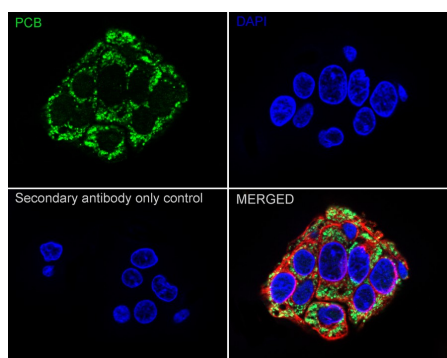
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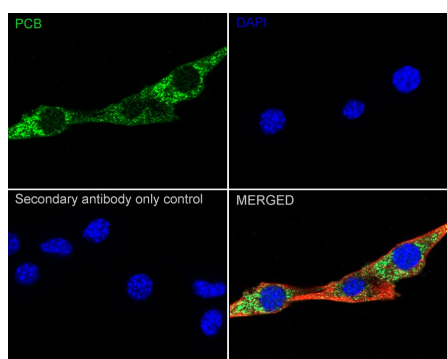
**Fig3:** Immunocytochemistry analysis of HepG2 cells labeling PCB with Rabbit anti-PCB antibody (ER64531) at 1/500 dilution.



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-PCB antibody (ER64531) at 1/500 dilution in 1% BSA in PBST overnight at 4 °C. 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 (HA601187, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

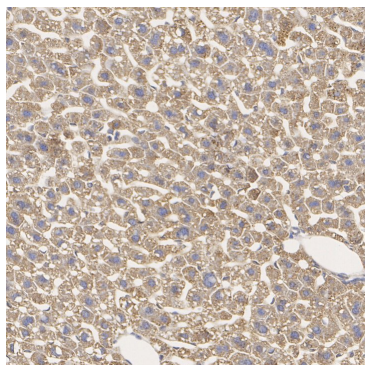
**Fig4:** Immunocytochemistry analysis of NIH/3T3 cells labeling PCB with Rabbit anti-PCB antibody (ER64531) at 1/500 dilution.



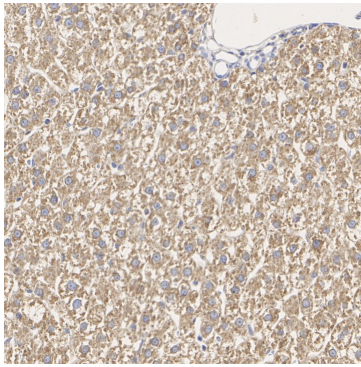
Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-PCB antibody (ER64531) at 1/500 dilution in 1% BSA in PBST overnight at 4 °C. 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 (HA601187, red) was stained at 1/100 dilution overnight at +4 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

**Fig5:** Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-PCB antibody (ER64531) at 1/1,000 dilution.

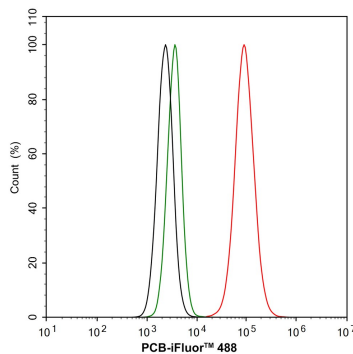


The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ER64531) at 1/1,000 dilution for 1 hour 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.



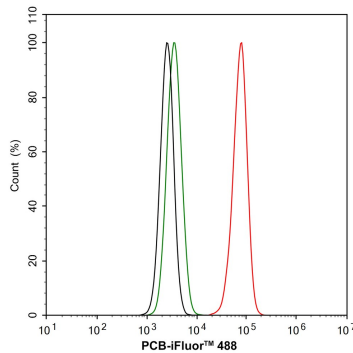
**Fig6:** Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-PCB antibody (ER64531) at 1/1,000 dilution.

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<sub>2</sub>O and PBS, and then probed with the primary antibody (ER64531) 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:** Flow cytometric analysis of HepG2 cells labeling PCB.

Cells were fixed and permeabilized. Then stained with the primary antibody (ER64531, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C 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°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).



**Fig8:** Flow cytometric analysis of NIH/3T3 cells labeling PCB.

Cells were fixed and permeabilized. Then stained with the primary antibody (ER64531, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C 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°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

**Note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

## 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

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