Anti-CPS1 Antibody [JB40-33]

ET7107-69

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, IP
Molecular Wt:	Predicted band size: 165 kDa
Clone number:	JB40-33

Description: The multicomplex protein, carbamoyl-phosphate synthetase-aspartate carbamoyl transferase-dihydro-orotase (CAD), consists of three distinct proteins, carbamoyl phosphate synthetase 2 (CPS2), aspartate transcarbamylase, and dihydro-orotase, which catalyze the second and third steps of pyrimidine biosynthesis. CAD is allosterically regulated by the phosphorylation of CPS2 by cyclic AMP-dependent protein kinase, and this activation enables CPS2 to catalyze the rate-limiting step of pyrimidine synthesis. CAD is expressed in brain and skeletal muscle. A related protein, carbamoyl phosphate synthetase 1 (CPS1) is expressed in liver. CPS1 catalyzes the rate-limiting step in the urea cycle, and deficiency of CPS1 is an autosomal recessive disorder that causes hyperammonemia.

Immunogen: Recombinant protein within Human CPS1 aa 82-204 / 1,500.

Positive control: HepG2 cell lysate, HeLa cell lysate, Mouse liver tissue lysate, Mouse small intestine tissue lysate, Rat liver tissue lysate, HepG2, human liver tissue, mouse liver tissue, rat liver tissue.

Subcellular location: Mitochondrion, Nucleus.

Database links: SwissProt: P31327 Human | Q8C196 Mouse | P07756 Rat

Recommended Dilutions:

WB	1:500-1:1,000
IF-Cell	1:50-1:200
IHC-P	1:50-1:200
IP	Use at an assay dependent concentration.

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

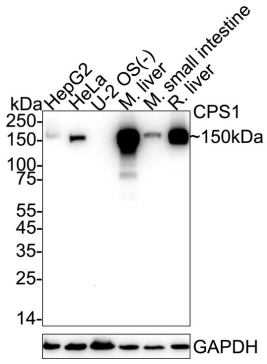

Fig1: Western blot analysis of CPS1 on different lysates with Rabbit anti-CPS1 antibody (ET7107-69) at 1/1,000 dilution.

Lane 1: HepG2 cell lysate (20 µg/Lane)
Lane 2: HeLa cell lysate (20 µg/Lane)
Lane 3: U-2 OS cell lysate (negative) (20 µg/Lane)
Lane 4: Mouse liver tissue lysate (20 µg/Lane)
Lane 5: Mouse small intestine tissue lysate (20 µg/Lane)
Lane 6: Rat liver tissue lysate (20 µg/Lane)

Predicted band size: 165 kDa
Observed band size: 150 kDa

Exposure time: 10 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 (ET7107-69) at 1/1,000 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.

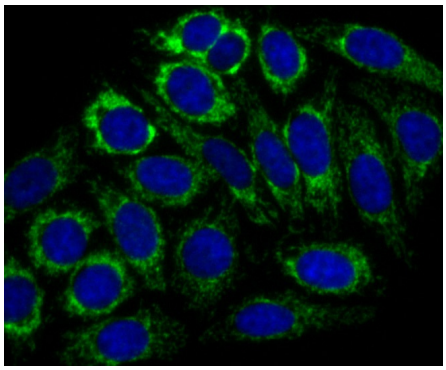

Fig2: ICC staining of CPS1 in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET7107-69, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

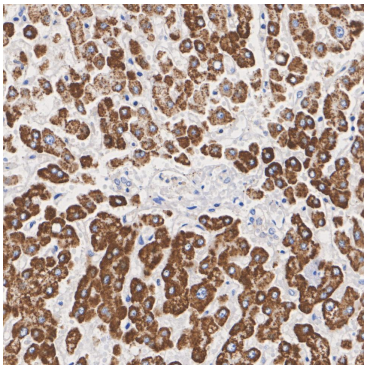

Fig3: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-CPS1 antibody (ET7107-69) at 1/200 dilution.

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 (ET7107-69) 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.

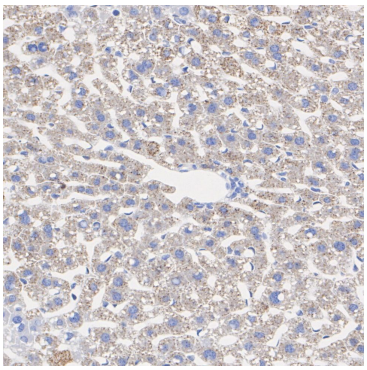

Fig4: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-CPS1 antibody (ET7107-69) at 1/200 dilution.

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 (ET7107-69) 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.

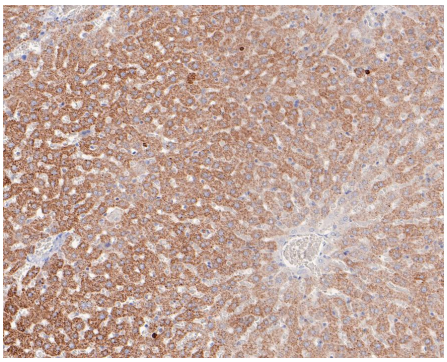

Fig5: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-CPS1 antibody (ET7107-69) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 2 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7107-69) 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.

Background References

1. Diez-Fernandez C et al. Molecular characterization of carbamoyl-phosphate synthetase (CPS1) deficiency using human recombinant CPS1 as a key tool. Hum Mutat 34:1149-1159 (2013).
2. Diez-Fernandez C et al. Understanding carbamoyl phosphate synthetase (CPS1) deficiency by using the recombinantly purified human enzyme: effects of CPS1 mutations that concentrate in a central domain of unknown function. Mol Genet Metab 112:123-132 (2014).