Anti-P4HB Antibody [JE54-98]

ET7110-92

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Monkey
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 57 kDa
Clone number:	JE54-98

Description: This gene encodes the beta subunit of prolyl 4-hydroxylase, a highly abundant multifunctional enzyme that belongs to the protein disulfide isomerase family. When present as a tetramer consisting of two alpha and two beta subunits, this enzyme is involved in hydroxylation of prolyl residues in preprocollagen. This enzyme is also a disulfide isomerase containing two thioredoxin domains that catalyze the formation, breakage and rearrangement of disulfide bonds. Other known functions include its ability to act as a chaperone that inhibits aggregation of misfolded proteins in a concentration-dependent manner, its ability to bind thyroid hormone, its role in both the influx and efflux of S-nitrosothiol-bound nitric oxide, and its function as a subunit of the microsomal triglyceride transfer protein complex.

Immunogen: Recombinant protein within Human P4HB aa 360-508 / 508.

Positive control: THP-1 cell lysate, HepG2 cell lysate, MCF7 cell lysate, NIH/3T3 cell lysate, COS-1 cell lysate, Mouse liver tissue lysate, Rat liver tissue lysate, HepG2, NIH/3T3, PC-12, human liver tissue, human placenta tissue, human pancreas tissue, mouse colon tissue, rat kidney tissue.

Subcellular location: Endoplasmic reticulum, endoplasmic reticulum lumen, cell membrane, melanosome.

Database links: SwissProt: P07237 Human | P09103 Mouse | P04785 Rat

Recommended Dilutions:

WB	1:2,000-1:10,000
IF-Cell	1:250
IHC-P	1:50-1:200
FC	1:1,000

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

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

Purity: Protein A affinity purified.

Images

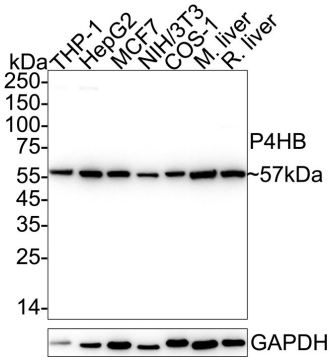

Fig1: Western blot analysis of P4HB on different lysates with Rabbit anti-P4HB antibody (ET7110-92) at 1/2,000 dilution.

Lane 1: THP-1 cell lysate (20 µg/Lane)
Lane 2: HepG2 cell lysate (20 µg/Lane)
Lane 3: MCF7 cell lysate (20 µg/Lane)
Lane 4: NIH/3T3 cell lysate (20 µg/Lane)
Lane 5: COS-1 cell lysate (20 µg/Lane)
Lane 6: Mouse liver tissue lysate (40 µg/Lane)
Lane 7: Rat liver tissue lysate (40 µg/Lane)

Predicted band size: 57 kDa
Observed band size: 57 kDa

Exposure time: 6 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 (ET7110-92) 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.

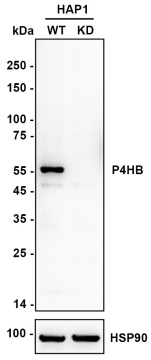

Fig2: Western blot analysis of P4HB on different lysates with Rabbit anti-P4HB antibody (ET7110-92) at 1/2,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-P4HB KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 57 kDa
Observed band size: 57 kDa

Exposure time: 120 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 (ET7110-92) at 1/2,000 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.

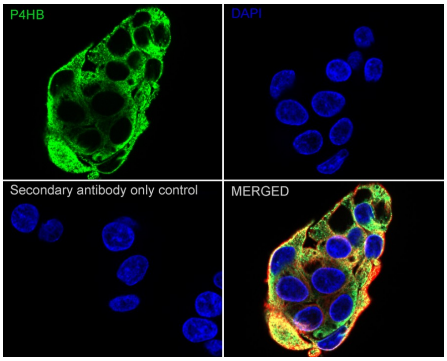

Fig3: Immunocytochemistry analysis of HepG2 cells labeling P4HB with Rabbit anti-P4HB antibody (ET7110-92) at 1/250 dilution.

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-P4HB antibody (ET7110-92) 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 (HA601187, 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.

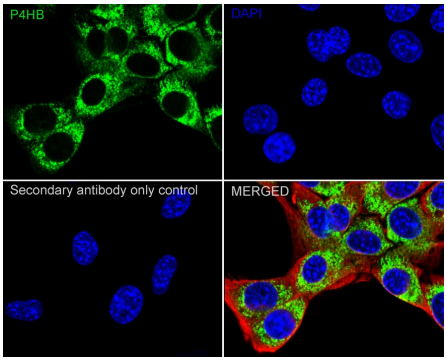

Fig4: Immunocytochemistry analysis of NIH/3T3 cells labeling P4HB with Rabbit anti-P4HB antibody (ET7110-92) at 1/250 dilution.

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-P4HB antibody (ET7110-92) 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 (HA601187, 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.

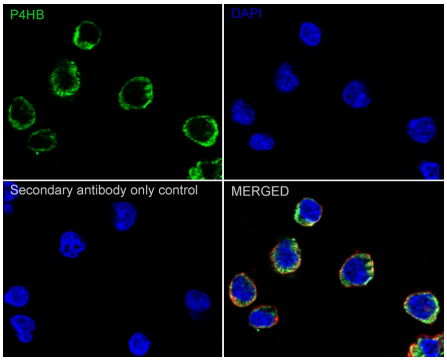

Fig5: Immunocytochemistry analysis of PC-12 cells labeling P4HB with Rabbit anti-P4HB antibody (ET7110-92) at 1/250 dilution.

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-P4HB antibody (ET7110-92) 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 (HA601187, 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.

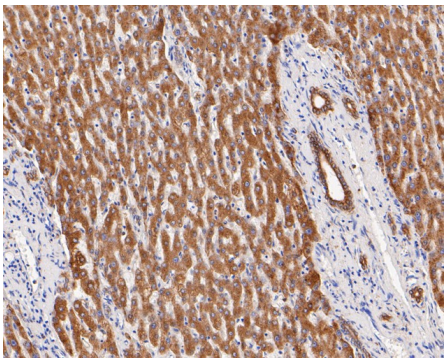

Fig6: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-P4HB antibody (ET7110-92) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4)) 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 (ET7110-92) at 1/200 dilution for 0.5 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.

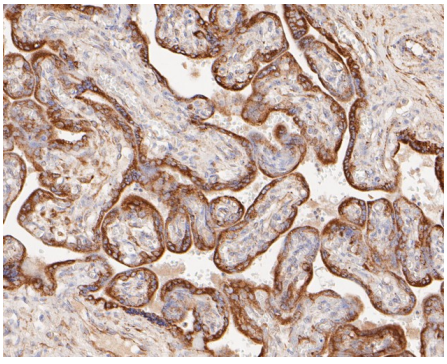

Fig7: Immunohistochemical analysis of paraffin-embedded human placenta tissue with Rabbit anti-P4HB antibody (ET7110-92) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4)) 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 (ET7110-92) at 1/50 dilution for 0.5 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.

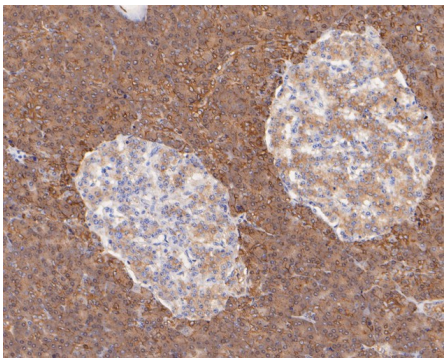

Fig8: Immunohistochemical analysis of paraffin-embedded human pancreas tissue with Rabbit anti-P4HB antibody (ET7110-92) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4)) 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 (ET7110-92) at 1/200 dilution for 0.5 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.

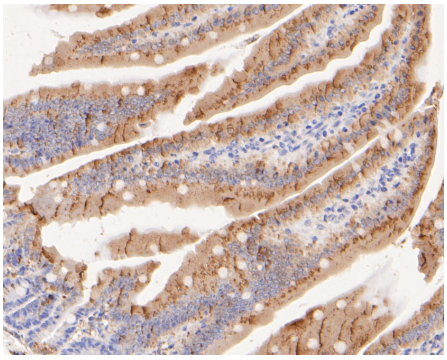

Fig9: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-P4HB antibody (ET7110-92) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4)) 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 (ET7110-92) at 1/200 dilution for 0.5 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.

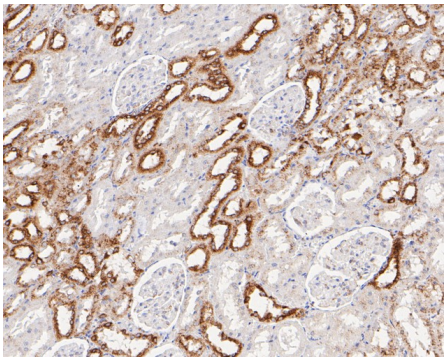

Fig10: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-P4HB antibody (ET7110-92) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4)) 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 (ET7110-92) at 1/50 dilution for 0.5 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.

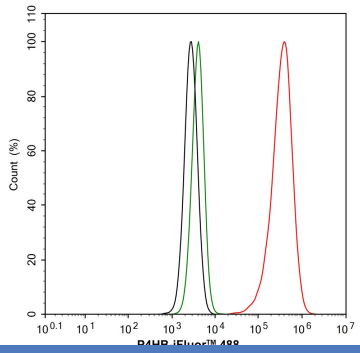

Fig11: Flow cytometric analysis of HepG2 cells labeling P4HB.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET7110-92, 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;

Background References

1. Williams SF. et. al. The History of GalaFLEX P4HB Scaffold. Aesthet Surg J. 2016 Nov.
2. Zou H. et. al. P4HB and PDIA3 are associated with tumor progression and therapeutic outcome of diffuse gliomas. Oncol Rep. 2018 Feb.