Anti-CLPP Antibody [JE40-00]

HA721503

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC
Molecular Wt:	Predicted band size: 30 kDa
Clone number:	JE40-00

Description: ATP-dependent Clp protease proteolytic subunit (ClpP) is an enzyme that in humans is encoded by the CLPP gene. This protein is an essential component to form the protein complex of Clp protease (Endopeptidase Clp). In bacteria, it was shown that ClpP is able to cleave full-length proteins without being associated with ClpA but the degradation is at a much slower rate. Fully functional Clp protease requires the participation of AAA+ ATPase. These ClpX chaperons recognize, unfold and transfer protein substrates to proteolytic core formed by ClpP tetradecamer. The proteolytic sites of ClpP subunits contain hydrophobic grooves which recruit substrate and host the catalytic triad Asp-His-Ser. In several bacteria, such as E. coli, proteins tagged with the SsrA peptide (ANDENYALAA) encoded by tmRNA are digested by Clp proteases. Proteases target damaged or misfolded proteins, transcription factors and signaling proteins in bacteria to coordinate complex cell responses and thus they have robust importance for the physiology and virulence of bacteria. In P. aeruginosa, ClpP1 is expressed constitutively throughout growth whereas ClpP2 expression is induced 10-fold in stationary phase. Quorum-sensing transcription factor LasR activates expression of ClpP2 in stationary phase. ClpP1 and ClpP2 have differential cleavage specificities which contributes to total peptidase activity of PaClpP17P27. Peptidase and protease action of PaClpP17P27 produces cleavage products that enhance biofilm formation in P. aeruginosa. The protein encoded by this gene belongs to the peptidase family S14 and hydrolyzes proteins into small peptides in the presence of ATP and magnesium. The protein is transported into mitochondrial matrix and is associated with the inner mitochondrial membrane.

Immunogen: Synthetic peptide within Human CLPP aa 228-277 / 277.

Positive control: HeLa cell lysate, K-562 cell lysate, SW620 cell lysate, Jurkat cell lysate, HepG2 cell lysate, MCF7 cell lysate, PC-12 cell lysate, mouse heart tissue lysate, rat heart tissue lysate, human colon carcinoma tissue, human prostate carcinoma tissue, mouse kidney tissue, rat kidney tissue, HeLa.

Subcellular location: Mitochondrion matrix.

Database links: SwissProt: Q16740 Human | O88696 Mouse
Entrez Gene: 301117 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IHC-P	1:1,000
IF-Cell	1:100
FC	1:500-1:1,000

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

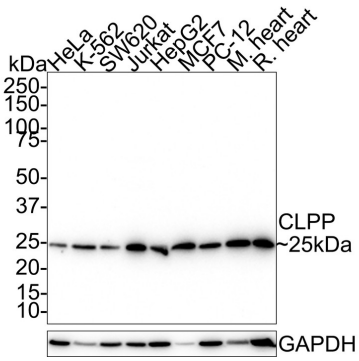

Fig1: Western blot analysis of CLPP on different lysates with Rabbit anti-CLPP antibody (HA721503) at 1/1,000 dilution.

Lane 1: HeLa cell lysate (20 µg/Lane)
Lane 2: K-562 cell lysate (20 µg/Lane)
Lane 3: SW620 cell lysate (20 µg/Lane)
Lane 4: Jurkat cell lysate (20 µg/Lane)
Lane 5: HepG2 cell lysate (20 µg/Lane)
Lane 6: MCF7 cell lysate (20 µg/Lane)
Lane 7: PC-12 cell lysate (20 µg/Lane)
Lane 8: Mouse heart tissue lysate (40 µg/Lane)
Lane 9: Rat heart tissue lysate (39 µg/Lane)

Predicted band size: 30 kDa
Observed band size: 25 kDa

Exposure time: 1 minute 55 seconds;

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 (HA721503) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

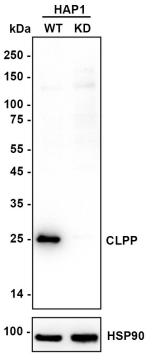

Fig2: Western blot analysis of CLPP on different lysates with Rabbit anti-CLPP antibody (HA721503) at 1/2,000 dilution.

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

Lysates/proteins at 10 µg/Lane.

Predicted band size: 30 kDa
Observed band size: 25 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 (HA721503) 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.

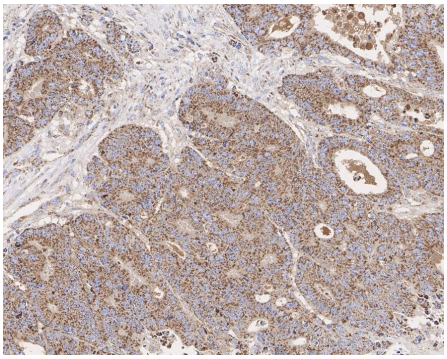

Fig3: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-CLPP antibody (HA721503) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA721503) 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.

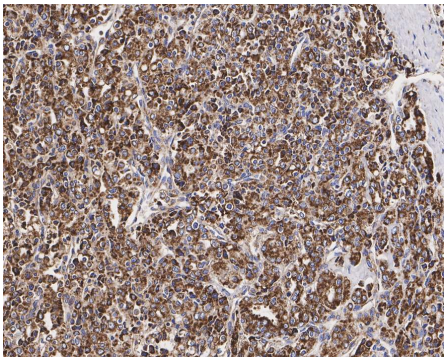

Fig4: Immunohistochemical analysis of paraffin-embedded human prostate carcinoma tissue with Rabbit anti-CLPP antibody (HA721503) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA721503) 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.

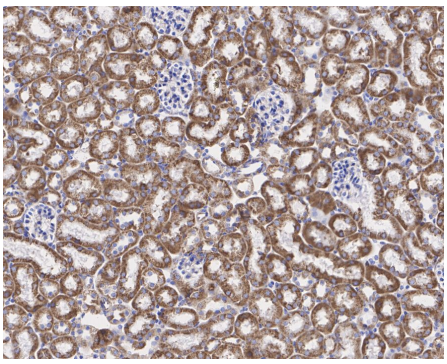

Fig5: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-CLPP antibody (HA721503) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA721503) 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.

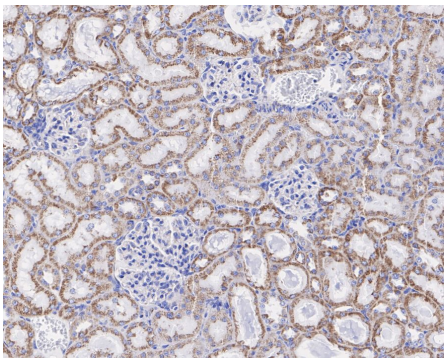

Fig6: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-CLPP antibody (HA721503) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (HA721503) 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.

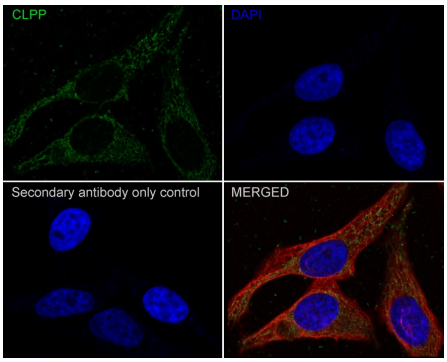

Fig7: Immunocytochemistry analysis of HeLa cells labeling CLPP with Rabbit anti-CLPP antibody (HA721503) at 1/100 dilution.

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-CLPP antibody (HA721503) 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.

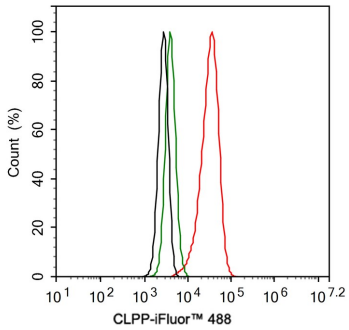

Fig8: Flow cytometric analysis of HeLa cells labeling CLPP.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721503, 1ug/ml) (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).

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

Background References