Anti-HSP60 Antibody [ST48-04] - BSA and Azide free

HA750175

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, IP
Molecular Wt:	Predicted band size: 61 kDa
Clone number:	ST48-04

Description: HSP60, also known as chaperonins (Cpn), is a family of heat shock proteins originally sorted by their 60kDa molecular mass. They prevent misfolding of proteins during stressful situations such as high heat, by assisting protein folding. HSP60 belong to a large class of molecules that assist protein folding, called molecular chaperones. Newly made proteins usually must fold from a linear chain of amino acids into a three-dimensional tertiary structure. The energy to fold proteins is supplied by non-covalent interactions between the amino acid side chains of each protein, and by solvent effects. Most proteins spontaneously fold into their most stable three-dimensional conformation, which is usually also their functional conformation, but occasionally proteins mis-fold. Molecular chaperones catalyze protein refolding by accelerating partial unfolding of misfolded proteins, aided by energy supplied by the hydrolysis of adenosine triphosphate (ATP). Chaperonin proteins may also tag misfolded proteins to be degraded.

Immunogen: Synthetic peptide within Human Hsp60 aa 421-457 / 573.

Positive control: HeLa cell lysate, HepG2 cell lysate, SW480 cell lysate, Jurkat cell lysate, PANC-1 cell lysate, F9 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, HeLa, NIH/3T3, PC-12, human kidney tissue, human lung cancer tissue, mouse kidney tissue, rat kidney tissue.

Subcellular location: Mitochondrion matrix.

Database links: SwissProt: P10809 Human | P63038 Mouse | P63039 Rat

Recommended Dilutions:

WB	1:20,000-1:100,000
IHC-P	1:1,000
IF-Cell	1:500-1:1,000
IP	1-2μg/sample

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

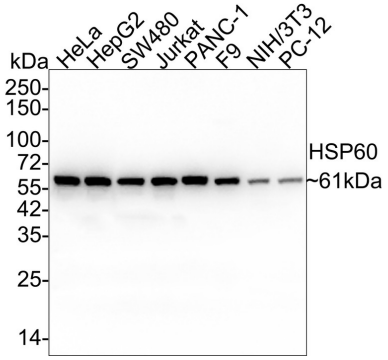

Fig1: Western blot analysis of HSP60 on different lysates with Rabbit anti-HSP60 antibody (HA750175) at 1/100,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HepG2 cell lysate
Lane 3: SW480 cell lysate
Lane 4: Jurkat cell lysate
Lane 5: PANC-1 cell lysate
Lane 6: F9 cell lysate
Lane 7: NIH/3T3 cell lysate
Lane 8: PC-12 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 61 kDa
Observed band size: 61 kDa

Exposure time: 46 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 (HA750175) at 1/100,000 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.

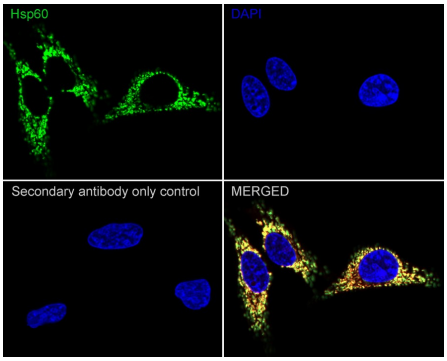

Fig2: Immunocytochemistry analysis of HeLa cells labeling HSP60 with Rabbit anti-HSP60 antibody (HA750175) at 1/1,000 dilution.

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-HSP60 antibody (HA750175) at 1/1,000 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. Counterstained with Mitotracker. Nuclear DNA was labelled in blue with DAPI.

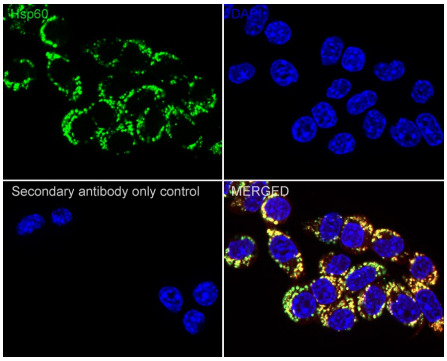

Fig3: Immunocytochemistry analysis of NIH3T3 cells labeling HSP60 with Rabbit anti-HSP60 antibody (HA750175) at 1/500 dilution.

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-HSP60 antibody (HA750175) at 1/500 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. Counterstained with Mitotracker. Nuclear DNA was labelled in blue with DAPI.

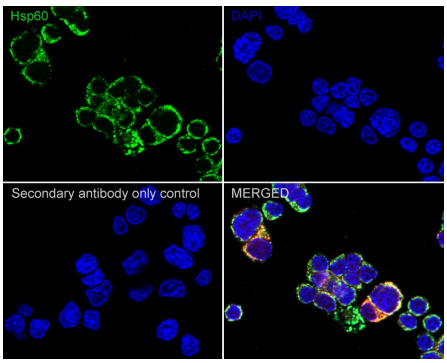

Fig4: Immunocytochemistry analysis of PC-12 cells labeling HSP60 with Rabbit anti-HSP60 antibody (HA750175) at 1/500 dilution.

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-HSP60 antibody (HA750175) at 1/500 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. Counterstained with Mitotracker. Nuclear DNA was labelled in blue with DAPI.

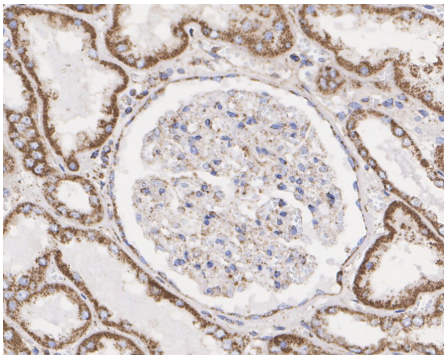

Fig5: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-HSP60 antibody (HA750175) at 1/1,000 dilution.

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 (HA750175) 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.

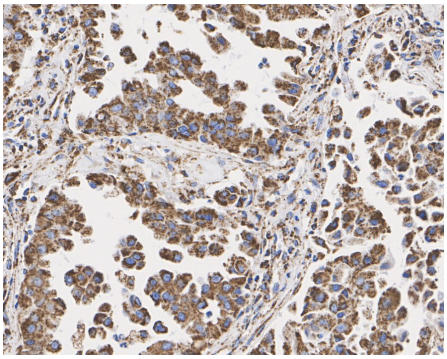

Fig6: Immunohistochemical analysis of paraffin-embedded human lung cancer tissue with Rabbit anti-HSP60 antibody (HA750175) at 1/1,000 dilution.

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 (HA750175) 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.

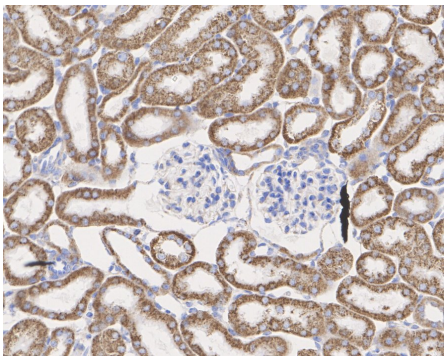

Fig7: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-HSP60 antibody (HA750175) at 1/1,000 dilution.

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 (HA750175) 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.

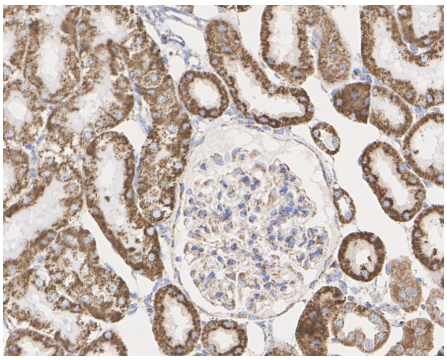

Fig8: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-HSP60 antibody (HA750175) at 1/1,000 dilution.

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 (HA750175) 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.

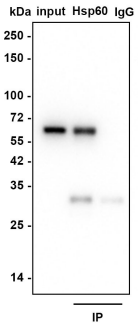

Fig9: HSP60 was immunoprecipitated in 0.2mg HeLa cell lysate with (HA750175) at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using (HA750175) at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/10,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: (HA750175) IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of (HA750175) in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 43 seconds

Background References

1. Suenaga S et al. Active hexose-correlated compound down-regulates HSP27 of pancreatic cancer cells, and helps the cytotoxic effect of gemcitabine. Anticancer Res 34:141-6 (2014).
2. Hauser DN et al. Post-translational decrease in respiratory chain proteins in the Polg mutator mouse brain. PLoS One 9:e94646 (2014).