Anti-Hsp40 Antibody [PSH12-10]

HA723426

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: 38 kDa
Clone number:	PSH12-10

Description: In molecular biology, chaperone DnaJ, also known as Hsp40 (heat shock protein 40 kDa), is a molecular chaperone protein. It is expressed in a wide variety of organisms from bacteria to humans. Molecular chaperones are a diverse family of proteins that function to protect proteins from irreversible aggregation during synthesis and in times of cellular stress. The bacterial molecular chaperone DnaK is an enzyme that couples cycles of ATP binding, hydrolysis, and ADP release by an N-terminal ATP-hydrolyzing domain to cycles of sequestration and release of unfolded proteins by a C-terminal substrate binding domain. Dimeric GrpE is the co-chaperone for DnaK, and acts as a nucleotide exchange factor, stimulating the rate of ADP release 5000-fold. DnaK is itself a weak ATPase; ATP hydrolysis by DnaK is stimulated by its interaction with another co-chaperone, DnaJ. Thus the co-chaperones DnaJ and GrpE are capable of tightly regulating the nucleotide-bound and substrate-bound state of DnaK in ways that are necessary for the normal housekeeping functions and stress-related functions of the DnaK molecular chaperone cycle.

Immunogen: Recombinant protein within human Hsp40 aa 1-340.

Positive control: HeLa cell lysate, HEK-293 cell lysate, HepG2 cell lysate, COS-1 cell lysate, NIH/3T3 cell lysate, C2C12 cell lysate, C6 cell lysate, HEK-293, C2C12, C6, human colon cancer tissue, human colon tissue, mouse colon tissue, rat colon tissue.

Subcellular location: Cytoplasm, Nucleus, nucleolus.

Database links: SwissProt: P25685 Human | Q9QYJ3 Mouse
Entrez Gene: 361384 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:100
IHC-P	1:200-1:1,000
FC	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% 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

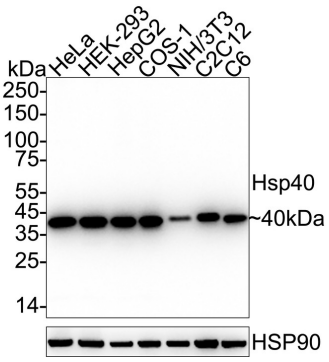

Fig1: Western blot analysis of Hsp40 on different lysates with Rabbit anti-Hsp40 antibody (HA723426) at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HEK-293 cell lysate
Lane 3: HepG2 cell lysate
Lane 4: COS-1 cell lysate
Lane 5: NIH/3T3 cell lysate
Lane 6: C2C12 cell lysate
Lane 7: C6 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 38 kDa
Observed band size: 40 kDa

Exposure time: 8 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 (HA723426) at 1/5,000 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.

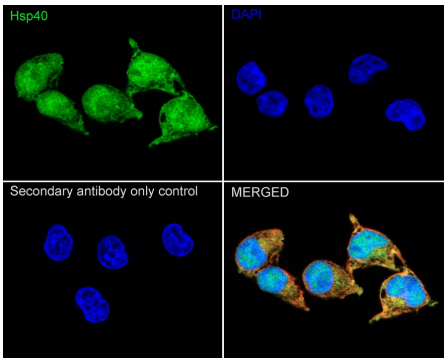

Fig2: Immunocytochemistry analysis of HEK-293 cells labeling Hsp40 with Rabbit anti-Hsp40 antibody (HA723426) at 1/100 dilution.

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-Hsp40 antibody (HA723426) 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 (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.

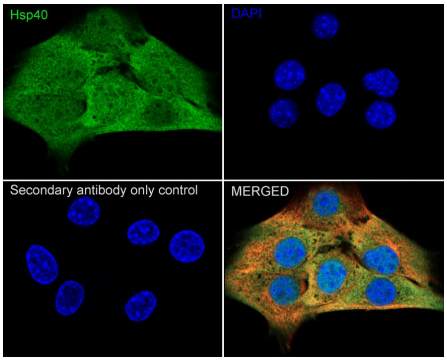

Fig3: Immunocytochemistry analysis of C2C12 cells labeling Hsp40 with Rabbit anti-Hsp40 antibody (HA723426) at 1/100 dilution.

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-Hsp40 antibody (HA723426) 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 (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.

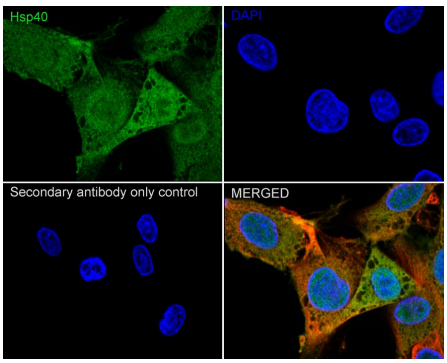

Fig4: Immunocytochemistry analysis of C6 cells labeling Hsp40 with Rabbit anti-Hsp40 antibody (HA723426) at 1/100 dilution.

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-Hsp40 antibody (HA723426) 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 (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.

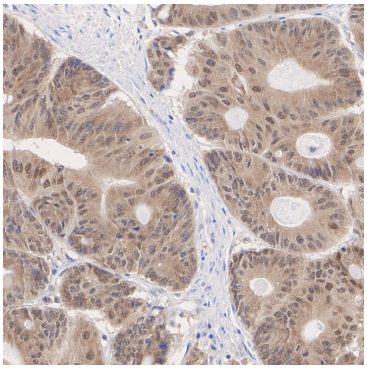

Fig5: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-Hsp40 antibody (HA723426) at 1/1,000 dilution.

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 (HA723426) 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.

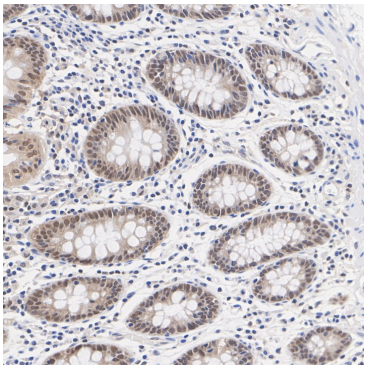

Fig6: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-Hsp40 antibody (HA723426) at 1/1,000 dilution.

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 (HA723426) 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.

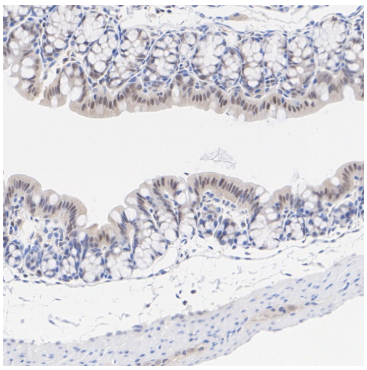

Fig7: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-Hsp40 antibody (HA723426) at 1/1,000 dilution.

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 (HA723426) 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.

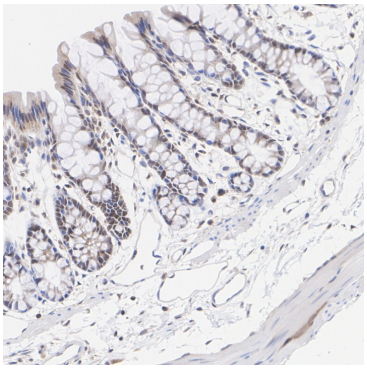

Fig8: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-Hsp40 antibody (HA723426) at 1/200 dilution.

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 (HA723426) 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.

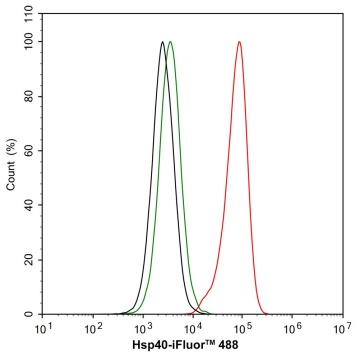

Fig9: Flow cytometric analysis of HEK-293 cells labeling Hsp40.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723426, 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; black).

Background References

1. He Y et al. The roles of HSP40/DNAJ protein family in neurodegenerative diseases. Zhejiang Da Xue Xue Bao Yi Xue Ban. 2022 Nov
2. Chang YL et al. The HSP40 family chaperone isoform DNAJB6b prevents neuronal cells from tau aggregation. BMC Biol. 2023 Dec