Anti-Hsp27 Antibody [JJ09-13]

ET1701-70

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 23 kDa
Clone number:	JJ09-13

Description: The heat shock proteins (HSPs) comprise a group of highly conserved, abundantly expressed proteins with diverse functions, including the assembly and sequestering of multiprotein complexes, transportation of nascent poly-peptide chains across cellular membranes and regulation of protein folding. Heat shock proteins (also known as molecular chaperones) fall into six general families: HSP 90, HSP 70, HSP 60, the low molecular weight HSPs, the immunophilins and the HSP 110 family. The low molecular weight family includes HSP 10, HSP 20, HSP 27, HSP 32 and HSP 40. HSP 27 is a constitutively expressed cytoplasmic protein that co-localizes to the nucleus upon stress induced by insult. Heat, cytokines and hormones are among the factors that stimulate the synthesis of HSP 27. In vitro, HSP 27 becomes highly phosphorylated following exposure to stress. The discovery that HSP 27 is regulated by hormones such as estrogen has led to studies establishing a relationship between HSP 27 and breast cancer.

Immunogen: Synthetic peptide within Human Hsp27 aa 127-176 / 205.

Positive control: Hela cell lysate, A549 cell lysate, Jurkat cell lysate, Hela, HepG2, human tonsil tissue, human colon carcinoma tissue, human breast carcinoma tissue.

Subcellular location: Cytoplasm, Cytoskeleton, Nucleus.

Database links: SwissProt: P04792 Human | P14602 Mouse | P42930 Rat

Recommended Dilutions:

WB	1:500-1:2,000
IF-Cell	1:100-1:500
IF-Tissue	1:100-1:500
IHC-P	1:50-1:200
FC	1:50-1:100
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: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

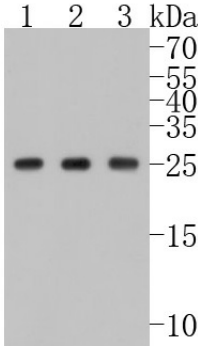

Fig1: Western blot analysis of Hsp27 on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1701-70, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: Hela cell lysate
Lane 2: A549 cell lysate
Lane 3: Jurkat cell lysate

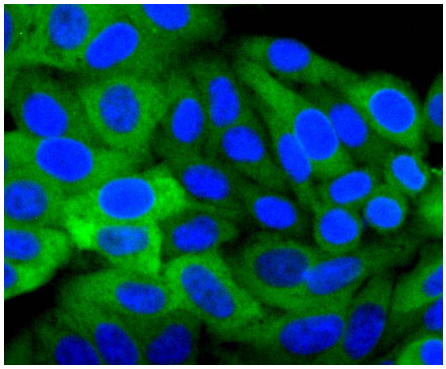

Fig2: ICC staining of Hsp27 in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1701-70, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

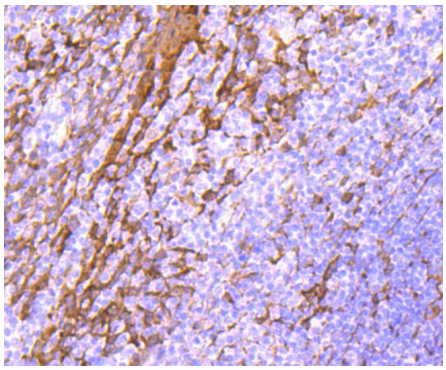

Fig3: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-Hsp27 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-70, 1/50) for 30 minutes 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.

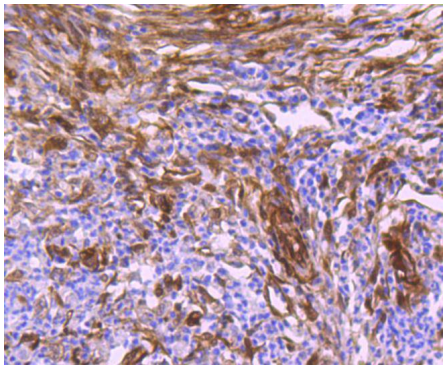

Fig4: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue using anti-Hsp27 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-70, 1/50) for 30 minutes 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.

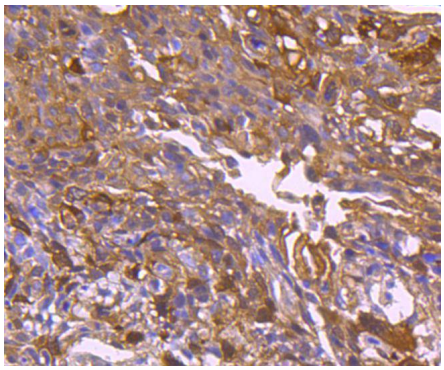

Fig5: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-Hsp27 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-70, 1/50) for 30 minutes 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.

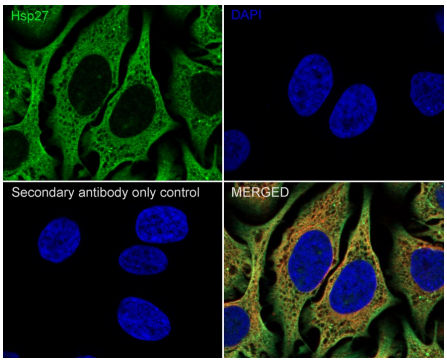

Fig6: Immunocytochemistry analysis of HeLa cells labeling Hsp27 with Rabbit anti-Hsp27 antibody (ET1701-70) at 1/100 dilution.

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-Hsp27 antibody (ET1701-70) 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.

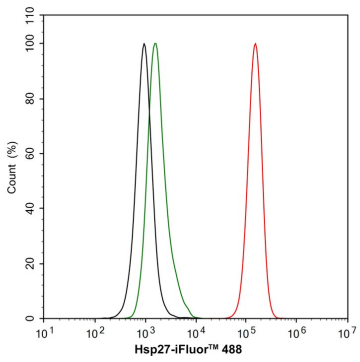

Fig7: Flow cytometric analysis of HeLa cells labeling Hsp27.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1701-70, 1µg/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

1. Zhai W et al. A1 adenosine receptor attenuates intracerebral hemorrhage-induced secondary brain injury in rats by activating the P38-MAPKAP2-Hsp27 pathway. Mol Brain 9:66 (2016).
2. White NM et al. Quantitative proteomic analysis reveals potential diagnostic markers and pathways involved in pathogenesis of renal cell carcinoma. Oncotarget 5:506-18 (2014).

Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation