Anti-Phospho-Hsp27 (S78) Antibody [JJ08-70] ET1701-19

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP
Molecular Wt:	Predicted band size: 23 kDa
Clone number:	JJ08-70

Description: Heat shock protein 27 (Hsp27) also known as heat shock protein beta-1 (HSPB1) is a protein that in humans is encoded by the HSPB1 gene. Hsp27 is a chaperone of the sHsp (small heat shock protein) group among α-crystallin, Hsp20, and others. The common functions of sHsps are chaperone activity, thermotolerance, inhibition of apoptosis, regulation of cell development, and cell differentiation. They also take part in signal transduction. The main function of Hsp27 is to provide thermotolerance in vivo, cytoprotection, and support of cell survival under stress conditions. A well documented function of Hsp27 is the interaction with actin and intermediate filaments. Another function of Hsp27 is the activation of the proteasome. Probably Hsp27 – among other chaperones – is involved in the process of cell differentiation.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser78 of human Hsp27.

Positive control: Human heart tissue lysate, human skeletal muscle tissue lysate, Hela, MCF-7, NIH/3T3, human breast carcinoma tissue, Hela cell lysate, Hela cell lysate treated with UV for 2 hours, human cervical carcinoma tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P04792 Human

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:400
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

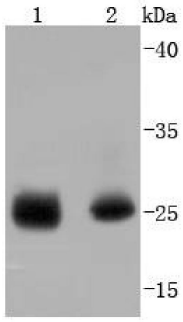

Fig1: Western blot analysis of Phospho-Hsp27 (S78) on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1701-19, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: Human heart tissue lysate
Lane 2: Human skeletal muscle tissue lysate

Predicted band size: 23 kDa
Observed band size: 27 kDa

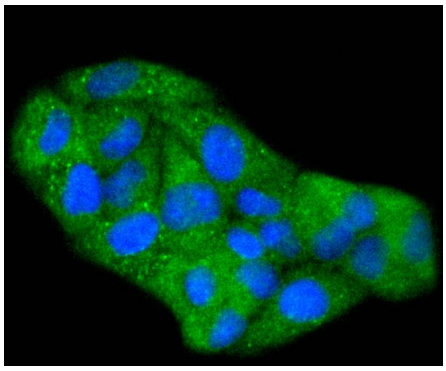

Fig2: ICC staining of Phospho-Hsp27 (S78) in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1701-19, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

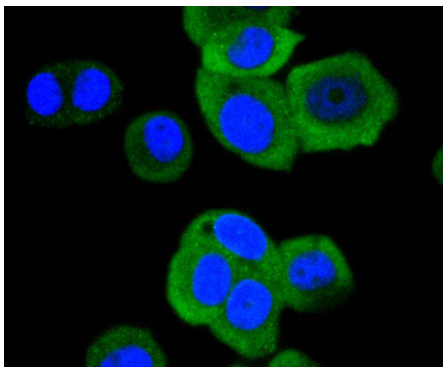

Fig3: ICC staining of Phospho-Hsp27 (S78) in MCF-7 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1701-19, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

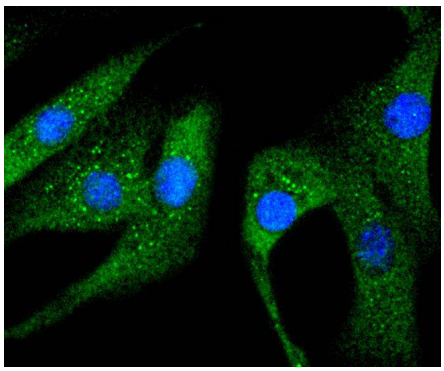

Fig4: ICC staining of Phospho-Hsp27 (S78) in NIH/3T3 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 10% negative goat serum for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1701-19, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

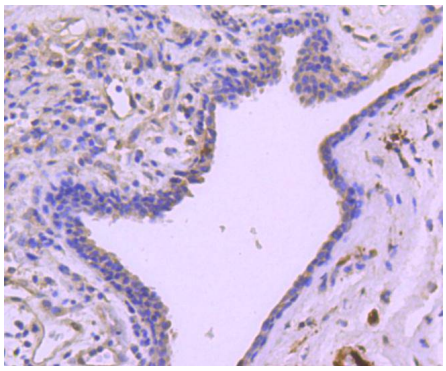

Fig5: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-Phospho-Hsp27 (S78) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-19, 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.

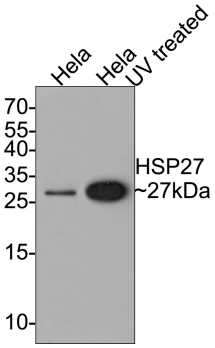

Fig6: Western blot analysis of Phospho-Hsp27 (S78) on different lysates with Rabbit anti-Phospho-Hsp27 (S78) antibody (ET1701-19) at 1/500 dilution.

Lane 1: Hela cell lysate
Lane 2: Hela cell lysate treated with UV for 2 hours

Lysates/proteins at 10 µg/Lane.

Predicted band size: 23 kDa
Observed band size: 27 kDa

Exposure time: 2 minutes;

15% 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 (ET1701-19) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

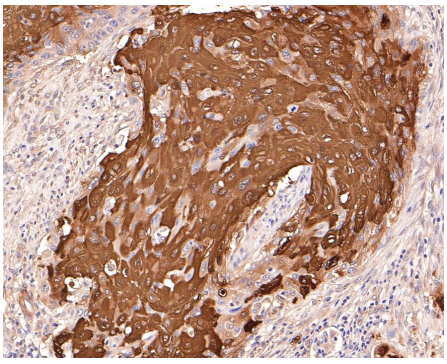

Fig7: Immunohistochemical analysis of paraffin-embedded human cervical carcinoma tissue with Rabbit anti-Phospho-Hsp27 (S78) antibody (ET1701-19) at 1/400 dilution.

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 (ET1701-19) at 1/400 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.

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).