Anti-Phospho-Hsp27 (S82) Antibody [SN06-73] ET1611-16

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IP, IF-Cell, FC
Molecular Wt:	Predicted band size: 23 kDa
Clone number:	SN06-73

Description: HSP 27 is a constitutively expressed cytoplasmic protein that co-localizes to the nucleus upon stress-induced insult. Heat shock, cytokines and hormones are among the factors that stimulate the synthesis of HSP 27. The intracellular concentration of the mammalian heat shock protein, HSP 27, increases several-fold after heat shock and other metabolic stresses, and is closely associated with the acquisition of thermotolerance. MAP kinase-activated protein kinase-2 phosphorylates HSP 27 on Serine residues Ser 15, Ser 78 and Ser 82, which are phosphorylated in vivo in response to growth factors and heat shock. Ser 15, Ser 78 and Ser 82 occur in the sequence motif RXXS, which is recognized by ribosomal protein S6 kinase II.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser82 of Human Hsp27 aa 50-99 / 205.

Positive control: Human skeletal muscle tissue lysate, MCF-7 cell lysate, MCF7.

Subcellular location: Cytoplasm, Nucleus.

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

Recommended Dilutions:

WB	1:500-1:2,000
IP	Use at an assay dependent concentration.
IF-Cell	1:100
FC	1:1,000

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

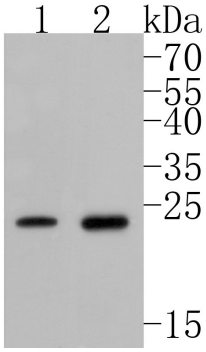

Fig1: Western blot analysis of Phospho-Hsp27 (S82) on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1611-16, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: human skeletal muscle tissue lysate
Lane 2: MCF-7 cell lysate

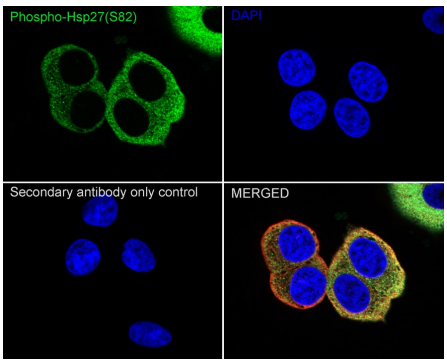

Fig2: Immunocytochemistry analysis of MCF7 cells labeling Phospho-Hsp27 (S82) with Rabbit anti-Phospho-Hsp27 (S82) antibody (ET1611-16) at 1/100 dilution.

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-Phospho-Hsp27 (S82) antibody (ET1611-16) 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.

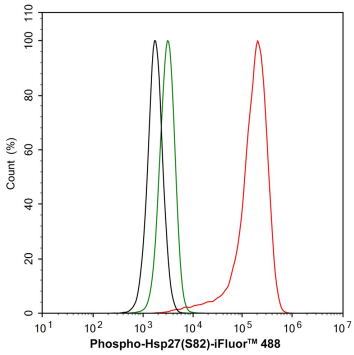

Fig3: Flow cytometric analysis of MCF7 cells labeling Phospho-Hsp27 (S82).

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1611-16, 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. Leja-Szpak, A. et al. 2015. Kynuramines induce overexpression of heat shock proteins in pancreatic cancer cells via 5-hydroxytryptamine and MT1/MT2 receptors. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society. 66: 711-8.
2. Shreeram, S. et al. 2006. Regulation of ATM/p53-dependent suppression of myc-induced lymphomas by Wip1 phosphatase. J Exp Med. 203(13): 2793-2799.