SANTA CRUZ BIOTECHNOLOGY, INC.

p-HSP 27 (D-3): sc-166694

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

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.

REFERENCES

1. Landry, J., et al. 1992. Human HSP 27 is phosphorylated at Serines 78 and 82 by heat shock and mitogen-activated kinases that recognize the same amino acid motif as S6 kinase II. J. Biol. Chem. 267: 794-803.
2. Stokoe, D., et al. 1992. Identification of MAPKAP kinase 2 as a major enzyme responsible for the phosphorylation of the small mammalian heat shock proteins. FEBS Lett. 313: 307-313.
3. Ciocca, D.R., et al. 1993. Biological and clinical implications of heat shock protein 27,000 (HSP 27): a review. J. Natl. Cancer Inst. 85: 1558-1570.
4. Freshney, N.W., et al. 1994. Interleukin-1 activates a novel protein kinase cascade that results in the phosphorylation of HSP 27. Cell 78: 1039-1049.
5. Mehlen, P., et al. 1995. Tumor necrosis factor α induces change in the phosphorylation, cellular localization and oligomerization of human HSP 27, a stress protein that confers cellular resistance to this cytokine. J. Cell. Biochem. 58: 248-259.

CHROMOSOMAL LOCATION

Genetic locus: HSPB1 (human) mapping to 7q11.23; Hspb1 (mouse) mapping to 5 G2.

SOURCE

p-HSP 27 (D-3) is a mouse monoclonal antibody specific for an epitope containing Ser 82 phosphorylated HSP 27 of human origin.

PRODUCT

Each vial contains 200 µg IgG_{2b} kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-166694 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% stabilizer protein).

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

APPLICATIONS

p-HSP 27 (D-3) is recommended for detection of Ser 82 phosphorylated HSP 27 of mouse, rat and human origin by Western Blotting (starting dilution 1:100, dilution range 1:5000-1:50000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500), immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

Suitable for use as control antibody for HSP 27 siRNA (h): sc-29350, HSP 27 siRNA (m): sc-35598, HSP 27 siRNA (r): sc-270545, HSP 27 shRNA Plasmid (h): sc-29350-SH, HSP 27 shRNA Plasmid (m): sc-35598-SH, HSP 27 shRNA Plasmid (r): sc-270545-SH, HSP 27 shRNA (h) Lentiviral Particles: sc-29350-V, HSP 27 shRNA (m) Lentiviral Particles: sc-35598-V and HSP 27 shRNA (r) Lentiviral Particles: sc-270545-V.

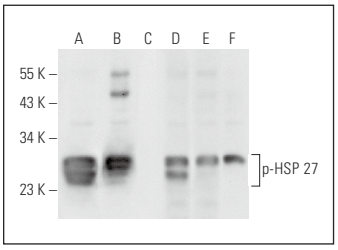
Molecular Weight of p-HSP 27: 27 kDa.

Positive Controls: HeLa whole cell lysate: sc-2200, ECV304 cell lysate: sc-2269 or HeLa + heat shock cell lysate: sc-2272.

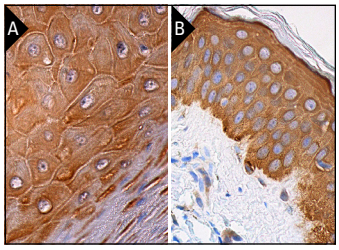
RECOMMENDED SUPPORT REAGENTS

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Lambda Phosphatase: sc-200312A and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850. 4) Immunohistochemistry: use m-IgGκ BP-HRP: sc-516102 with DAB, 50X: sc-24982 and Immunohistomount: sc-45086, or Organo/Limonene Mount: sc-45087.

DATA



Western blot analysis of HSP 27 phosphorylation in untreated (**A**,**D**), UV irradiated (**B**,**E**) and UV irradiated and lambda protein phosphatase treated (**C**,**F**) HeLa whole cell lysates. Antibodies tested include p-HSP 27 (D-3): sc-166694 (**A**,**B**,**C**) and HSP 27 (F-4): sc-13132 (**D**,**E**,**F**).



p-HSP 27 (D-3): sc-166694. Immunoperoxidase staining of formalin fixed, paraffin-embedded human oral mucosa tissue showing cytoplasmic staining of squamous epithelial cells (**A**) and human skin tissue showing cytoplasmic staining of keratinocytes, fibroblasts, Langerhans cells and melanocytes (**B**).

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.