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HSP 27 siRNA (h2): sc-44264

BACKGROUND

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 polypeptide 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.

REFERENCES

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2. Lemeaux, P.G., et al. 1978. Transient rates of synthesis of individual polypeptides in *E. coli* following temperature shifts. *Cell* 13: 427-434.
3. Kelley, P., et al. 1978. The effect of amino acid analogues and heat shock on gene expression in chicken embryo fibroblasts. *Cell* 15: 1277-1286.
4. Schlesinger, M.J., et al. 1982. Heat Shock: from Bacteria to Man: Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
5. Ciocca, D.R., et al. 1993. Biological and clinical implications of heat shock protein 27,000 (HSP 27). *J. Natl. Cancer Inst.* 85: 1558-1570.
6. Todd, M.J., et al. 1994. Dynamics of the chaperonin ATPase cycle: implications for facilitated protein folding. *Science* 265: 659-666.
7. 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.

CHROMOSOMAL LOCATION

Genetic locus: HSPB1 (human) mapping to 7q11.23.

PRODUCT

HSP 27 siRNA (h2) is a pool of 2 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HSP 27 shRNA Plasmid (h2): sc-44264-SH and HSP 27 shRNA (h2) Lentiviral Particles: sc-44264-V as alternate gene silencing products.

For independent verification of HSP 27 (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44264A and sc-44264B.

PROTOCOLS

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

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

HSP 27 siRNA (h2) is recommended for the inhibition of HSP 27 expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

HSP 27 (F-4): sc-13132 is recommended as a control antibody for monitoring of HSP 27 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor HSP 27 gene expression knockdown using RT-PCR Primer: HSP 27 (h2)-PR: sc-44264-PR (20 μ l, 466 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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