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HSP 27 shRNA (h2) Lentiviral Particles: sc-44264-V

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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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.
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CHROMOSOMAL LOCATION

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

PRODUCT

HSP 27 shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle' Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see HSP 27 siRNA (h2): sc-44264 and HSP 27 shRNA Plasmid (h2): sc-44264-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

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

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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