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HSP 70 siRNA (h2): sc-44196

BACKGROUND

The HSP 70 family is composed of four highly conserved proteins: HSP 70, HSC 70, GRP 75 and GRP 78. These proteins serve a variety of roles: they act as molecular chaperones facilitating the assembly of multi-protein complexes, participate in the translocation of polypeptides across cell membranes and to the nucleus and aid in the proper folding of nascent polypeptide chains. All members of the family, except HSP 70, are constitutively expressed in primate cells. HSP 70 expression is strongly induced in response to heat stress. HSP 70 and HSC 70 play key roles in the cytosolic endoplasmic reticulum and mitochondrial import machinery and are found in both the cytosol and nucleus of mammalian cells. Both HSP 70 and HSC 70 are involved in the chaperoning of nascent polypeptide chains and in protecting cells against the accumulation of improperly folded proteins. GRP 78 is localized in the endoplasmic reticulum, where it receives imported secretory proteins and is involved in the folding and translocation of nascent peptide chains. GRP 75 expression is restricted to the mitochondrial matrix and aids in the translocation and folding of nascent polypeptide chains of both nuclear and mitochondrial origin. GRP 75 and GRP 78 are unresponsive to heat stress and are induced by glucose deprivation. It has been postulated that members of the HSP 70 family act as force-generating motors, relying on the hydrolysis of ATP for their activity.

REFERENCES

- Martin, J., et al. 1992. Prevention of protein denaturation under heat stress by the chaperonin HSP 60. *Science* 258: 995-998.
- Hatayama, T., et al. 1992. Effects of low culture temperature on the induction of HSP 70 mRNA and the accumulation of HSP 70 and HSP 105 in mouse FM3A cells. *J. Biochem.* 111: 484-490.
- Haas, I.G. 1995. Protein-mediated protein maturation in eukaryotes. *FEBS Lett.* 369: 72-75.
- Glick, B.S. 1995. Can HSP 70 proteins act as force-generating motors? *Cell* 80: 11-14.
- Csermely, P., et al. 1995. Autophosphorylation of GRP94 (endoplasmic reticulum chaperone). *J. Biol. Chem.* 270: 6381-6388.

PRODUCT

HSP 70 siRNA (h2) is a pool of 3 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 70 shRNA Plasmid (h2): sc-44196-SH and HSP 70 shRNA (h2) Lentiviral Particles: sc-44196-V as alternate gene silencing products.

For independent verification of HSP 70 (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-44196A, sc-44196B and sc-44196C.

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 70 siRNA (h2) is recommended for the inhibition of HSP 70 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 70 (F-3): sc-373867 is recommended as a control antibody for monitoring of HSP 70 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 70 gene expression knockdown using RT-PCR Primer: HSP 70 (h2)-PR: sc-44196-PR (20 μ l, 416 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.