SANTA CRUZ BIOTECHNOLOGY, INC.

Hip siRNA (m): sc-40684

BACKGROUND

The HSP 70 family is comprised of four highly conserved proteins: HSP 70, HSC 70, GRP 75 and GRP 78. These proteins serve a variety functions as molecular chaperones and aide in the assembly of multi-protein complexes. In addition to these specialized functions, the HSP 70 family may play a more general role in stabilizing protein conformation and preventing protein aggregation. HSC 70 in the mitochondrial and endoplasmic reticulum acts as ATP-driven, force generating motors that translocate proteins across organelle membranes. An HSC 70-interacting protein designated Hip, has been identified as a cochaperone in the HSC 70/HSP 40 reaction cycle. One Hip oligomer binds the ATPase domains of at least two HSC 70 molecules. This association is dependent on the activation of the HSC 70 ATPase by HSP 40. Hip functions to stabilize HSC 70 in the ADP-bound state which has high affinity for substrate protein. Through its own chaperoning activity, Hip may contribute to the substrate specificity of the HSC 70 complex.

REFERENCES

1. Martin, J., et al. 1992. Prevention of protein denaturation under heat stress by the chaperonin HSP 60. Science 258: 995-998.
2. 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.
3. Haas, I.G. 1995. Protein-mediated protein maturation in eukaryotes. FEBS Lett. 369: 72-75.
4. Glick, B.S. 1995. Can HSP 70 proteins act as force-generating motors? Cell 80: 11-14.
5. Höhfeld, J., et al. 1995. Hip, a novel cochaperone involved in the eukaryotic HSC 70/HSP 40 reaction cycle. Cell 83: 589-598.
6. Csermely, P., et al. 1995. Autophosphorylation of grp94 (endoplasmin). J. Biol. Chem. 270: 6381-6388.
7. Hatayama, T., et al. 1992. Effects of low culture temperature on the induction of HSC 70 mRNA and the accumulation of HSC 70 and HSC 105 in mouse FM3A cells. J. Biochem. 111: 484-490.

CHROMOSOMAL LOCATION

Genetic locus: St13 (mouse) mapping to 15 E1.

PRODUCT

Hip siRNA (m) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Hip shRNA Plasmid (m): sc-40684-SH and Hip shRNA (m) Lentiviral Particles: sc-40684-V as alternate gene silencing products.

For independent verification of Hip (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-40684A, sc-40684B and sc-40684C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Hip siRNA (m) is recommended for the inhibition of Hip expression in mouse 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 µM in 66 µl. 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

Hip (11A6): sc-136175 is recommended as a control antibody for monitoring of Hip 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-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, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Hip gene expression knockdown using RT-PCR Primer: Hip (m)-PR: sc-40684-PR (20 µl, 438 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.

PROTOCOLS

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