

HSP 40 siRNA (h): sc-35599

BACKGROUND

Heat shock protein 40 (HSP 40) family proteins bind to HSP 70 through their J-domain and regulate the function of HSP 70 by stimulating HSP 70 ATPase activity. HSP 40, also known as DnaJ, functions together with DnaK (HSP 70) and GrpE as a molecular chaperone, involving them in assembly and disassembly of protein complexes, protein folding, renaturation of denatured proteins, prevention of protein aggregation and protein export. HSP 40 stimulates the association between HSC 70 and HIP and translocates rapidly from the cytoplasm to the nuclei, and especially to the nucleoli, upon heat shock. There are five known HSP 40 family proteins.

CHROMOSOMAL LOCATION

Genetic locus: DNAJB1 (human) mapping to 19p13.12.

PRODUCT

HSP 40 siRNA (h) 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 40 shRNA Plasmid (h): sc-35599-SH and HSP 40 shRNA (h) Lentiviral Particles: sc-35599-V as alternate gene silencing products.

For independent verification of HSP 40 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35599A, sc-35599B and sc-35599C.

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 40 siRNA (h) is recommended for the inhibition of HSP 40 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 40 (B-3): sc-398766 is recommended as a control antibody for monitoring of HSP 40 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 40 gene expression knockdown using RT-PCR Primer: HSP 40 (h)-PR: sc-35599-PR (20 μ l, 483 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Yun, C.H., et al. 2010. Geldanamycin inhibits TGF- β signaling through induction of HSP 70. Arch. Biochem. Biophys. 495: 8-13.
2. Lopes-Pacheco, M., et al. 2015. Combination of correctors rescue Δ F508-CFTR by reducing its association with HSP 40 and HSP 27. J. Biol. Chem. 290: 25636-25645.
3. Hiraki, M., et al. 2015. Small-molecule reactivation of mutant p53 to wild-type-like p53 through the p53-HSP 40 regulatory axis. Chem. Biol. 22: 1206-1216.
4. Chaudhary, P., et al. 2016. HSP 70 binding protein 1 (HspBP1) suppresses HIV-1 replication by inhibiting NF κ B mediated activation of viral gene expression. Nucleic Acids Res. 44: 1613-1629.
5. Lopes-Pacheco, M., et al. 2016. Correctors rescue CFTR mutations in nucleotide-binding domain 1 (NBD1) by modulating proteostasis. Chembiochem 17: 493-505.
6. Wang, P., et al. 2017. Acetylation-induced TDP-43 pathology is suppressed by an HSF1-dependent chaperone program. Nat. Commun. 8: 82.
7. Silva, G., et al. 2018. *Trans*-chalcone increases p53 activity via DNAJB1/HSP40 induction and CRM1 inhibition. PLoS ONE 13: e0202263.

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.