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HspBP1 siRNA (m): sc-45315

BACKGROUND

Hsp70-interacting protein (HspBP1) belongs to a family of eukaryotic proteins identified as nucleotide exchange factors for Hsp70, which exhibit varying degrees of compartment and species specificity. HspBP1 interferes with the CHIP-induced degradation of immature forms of the cystic fibrosis transmembrane conductance regulator (CFTR) and stimulates CFTR maturation. HspBP1 binds to Hsp70, inhibits its activity and promotes dissociation of nucleotides from the Hsp70 ATPase domain. It is a protein mainly expressed in heart and skeletal muscle.

REFERENCES

1. Raynes, D.A., et al. 2000. Isolation and characterization of isoforms of HspBP1, inhibitors of HSP 70. *Biochim. Biophys. Acta* 1490: 203-207.
2. Kabani, M., et al. 2002. HspBP1, a homologue of the yeast Fes1 and SIs1 proteins, is an Hsc70 nucleotide exchange factor. *FEBS Lett.* 531: 339-342.
3. McLellan, C.A., et al. 2003. HspBP1, an HSP 70 cochaperone, has two structural domains and is capable of altering the conformation of the HSP 70 ATPase domain. *J. Biol. Chem.* 278: 19017-19022.
4. Raynes, D.A., et al. 2003. Increased expression of the Hsp70 cochaperone HspBP1 in tumors. *Tumour Biol.* 24: 281-285.
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6. Alberti, S. et al. 2004. The cochaperone HspBP1 inhibits the CHIP ubiquitin ligase and stimulates the maturation of the cystic fibrosis transmembrane conductance regulator. *Mol. Biol. Cell* 15: 4003-4010.
7. Shomura, Y., et al. 2005. Regulation of Hsp70 function by HspBP1: structural analysis reveals an alternate mechanism for Hsp70 nucleotide exchange. *Mol. Cell* 17: 367-379.
8. Papp, D., et al. 2005. Development of a sensitive assay for the measurement of antibodies against heat shock protein binding protein 1 (HspBP1): increased levels of anti-HspBP1 IgG are prevalent in HIV infected subjects. *J. Med. Virol.* 76: 464-469.

CHROMOSOMAL LOCATION

Genetic locus: Hspbp1 (mouse) mapping to 7 A1.

PRODUCT

HspBP1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HspBP1 shRNA Plasmid (m): sc-45315-SH and HspBP1 shRNA (m) Lentiviral Particles: sc-45315-V as alternate gene silencing products.

For independent verification of HspBP1 (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-45315A, sc-45315B and sc-45315C.

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

HspBP1 siRNA (m) is recommended for the inhibition of HspBP1 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 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

HspBP1 (F-11): sc-390467 is recommended as a control antibody for monitoring of HspBP1 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 IgM-HRP: sc-2064 (dilution range: 1:500-1:5,000), TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgM-FITC: sc-2082 (dilution range: 1:100-1:400) or goat anti-mouse IgM-TR: sc-2983 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor HspBP1 gene expression knockdown using RT-PCR Primer: HspBP1 (m)-PR: sc-45315-PR (20 μ l). 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.