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Diagnostik & molekulare Diagnostik



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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic in



HspBP1 shRNA (h) Lentiviral Particles: sc-45314-V



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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.
- 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.
- 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.
- Tanimura, S., et al. 2004. Heat shock protein 70 binding protein 1 induces enhanced apoptotic response against anticancer drugs in tumor cells. Nippon Rinsho 62: 1291-1296.
- 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.

CHROMOSOMAL LOCATION

Genetic locus: HSPBP1 (human) mapping to 19q13.42.

PRODUCT

HspBP1 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 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 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see HspBP1 siRNA (h): sc-45314 and HspBP1 shRNA Plasmid (h): sc-45314-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.

PROTOCOLS

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

APPLICATIONS

HspBP1 shRNA (h) Lentiviral Particles is recommended for the inhibition of HspBP1 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0 x 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

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 (h)-PR: sc-45314-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

Santa Cruz Biotechnology, Inc. 1.800.457.3801 831.457.3801 Fax 831.457.3801 Europe +00800 4573 8000 49 6221 4503 0 www.scbt.com