SANTA CRUZ BIOTECHNOLOGY, INC.

Bag-3 siRNA (m): sc-72603

BACKGROUND

The Bag family of proteins are characterized by the presence of a 45 amino acid Bag domain through which they bind with high affinity to the ATPase domain of HSP 70, thereby negatively regulating HSP 70 chaperone activity. Bag-3 (Bcl-2-associated athanogene 3), also known as BIS or CAIR-1, is a 575 amino acid protein that contains one C-terminal Bag domain and two N-terminal WW domains. Like other members of the Bag family, Bag-3 functions to inhibit the chaperone activity of HSP 70, specifically by promoting the release of HSP 70-bound substrates. Additionally, Bag-3 exhibits anti-apoptotic activity via cell cycle control, suggesting a possible role for Bag-3 in tumor progression. The gene encoding Bag-3 maps to human chromosome 10, which houses over 1,200 genes and comprises nearly 4.5% of the human genome.

REFERENCES

1. Takayama, S., et al. 1999. An evolutionarily conserved family of HSP 70/HSC 70 molecular chaperone regulators. J. Biol. Chem. 274: 781-786.
2. Liao, Q., et al. 2001. The anti-apoptotic protein Bag-3 is overexpressed in pancreatic cancer and induced by heat stress in pancreatic cancer cell lines. FEBS Lett. 503: 151-157.
3. Iwasaki, M., et al. 2007. Bag-3 regulates motility and adhesion of epithelial cancer cells. Cancer Res. 67: 10252-10259.
4. Chiappetta, G., et al. 2007. The antiapoptotic protein Bag-3 is expressed in thyroid carcinomas and modulates apoptosis mediated by tumor necrosis factor-related apoptosis-inducing ligand. J. Clin. Endocrinol. Metab. 92: 1159-1163.
5. Carra, S., et al. 2008. HspB8 and Bag-3: a new chaperone complex targeting misfolded proteins to macroautophagy. Autophagy 4: 237-239.
6. Wang, H.Q., et al. 2008. Transcriptional upregulation of Bag-3 upon proteasome inhibition. Biochem. Biophys. Res. Commun. 365: 381-385.
7. Carra, S., et al. 2008. HspB8 chaperone activity toward poly(Q)-containing proteins depends on its association with Bag-3, a stimulator of macroautophagy. J. Biol. Chem. 283: 1437-1444.
8. Franceschelli, S., et al. 2008. Bag-3 gene expression is regulated by heat shock factor 1. J. Cell. Physiol. 215: 575-577.

CHROMOSOMAL LOCATION

Genetic locus: Bag3 (mouse) mapping to 7 F3.

PRODUCT

Bag-3 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 Bag-3 shRNA Plasmid (m): sc-72603-SH and Bag-3 shRNA (m) Lentiviral Particles: sc-72603-V as alternate gene silencing products.

For independent verification of Bag-3 (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-72603A, sc-72603B and sc-72603C.

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

Bag-3 siRNA (m) is recommended for the inhibition of Bag-3 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

Bag-3 (19): sc-136467 is recommended as a control antibody for monitoring of Bag-3 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 Bag-3 gene expression knockdown using RT-PCR Primer: Bag-3 (m)-PR: sc-72603-PR (20 µl). 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.