SANTA CRUZ BIOTECHNOLOGY, INC.

Bag-5 siRNA (h): sc-72604

BACKGROUND

Bag-5 (Bcl-2-associated athanogene 5), also known as Bag family molecular chaperone regulator 5, is a member of the Bag family of proteins and contains four Bag domains. Via their Bag domain, Bag proteins bind with high affinity to the HSC 70/HSP 70 ATPase domain, regulating chaperone activity and apoptosis. Bag-5 is a component of the HSP 70/Parkin complex and acts to inhibit Parkin E3 ubiquitin ligase activity and HSP 70 chaperone activity. In this complex, Bag-5 directly interacts with the ATPase domain of HSP 70 and the N-terminal linker region of Parkin. Bag-5 expression is induced upon dopaminergic neuron injury and functions to sensitize the neurons to injury-induced cell death. In addition, Bag-5 may be a useful target in therapies for neurodegenerative diseases such as Parkinson's disease which is caused by a mutation in the gene encoding Parkin.

REFERENCES

1. Höhfeld, J., et al. 1997. GrpE-like regulation of the HSC 70 chaperone by the anti-apoptotic protein BAG-1. EMBO J. 16: 6209-6216.
2. Takayama, S., et al. 1999. An evolutionarily conserved family of HSP 70/HSC 70 molecular chaperone regulators. J. Biol. Chem. 274: 781-786.
3. Briknarová, K., et al. 2002. BAG4/SODD protein contains a short Bag domain. J. Biol. Chem. 277: 31172-31178.
4. Kalia, S.K., et al. 2004. Bag-5 inhibits Parkin and enhances dopaminergic neuron degeneration. Neuron 44: 931-945.
5. Esser, C., et al. 2004. Cooperation of molecular chaperones with the ubiquitin/proteasome system. Biochim. Biophys. Acta 1695: 171-188.
6. Liman, J., et al. 2005. Interaction of BAG1 and HSP 70 mediates neuroprotectivity and increases chaperone activity. Mol. Cell. Biol. 25: 3715-3725.
7. Wada, S., et al. 2006. A genomewide analysis of genes for the heat shock protein 70 chaperone system in the ascidian *Ciona intestinalis*. Cell Stress Chaperones 11: 23-33.

CHROMOSOMAL LOCATION

Genetic locus: BAG5 (human) mapping to 14q32.33.

PRODUCT

Bag-5 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Bag-5 shRNA Plasmid (h): sc-72604-SH and Bag-5 shRNA (h) Lentiviral Particles: sc-72604-V as alternate gene silencing products.

For independent verification of Bag-5 (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-72604A, sc-72604B and sc-72604C.

PROTOCOLS

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

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-5 siRNA (h) is recommended for the inhibition of Bag-5 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 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-5 (F-9): sc-390832 is recommended as a control antibody for monitoring of Bag-5 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-5 gene expression knockdown using RT-PCR Primer: Bag-5 (h)-PR: sc-72604-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Guo, K., et al. 2015. Bag5 protects neuronal cells from amyloid β-induced cell death. J. Mol. Neurosci. 55: 815-820.

RESEARCH USE

For research use only, not for use in diagnostic procedures.