

Bag-1 siRNA (r): sc-61877

BACKGROUND

The Bcl-2 family of proteins is characterized by its ability to modulate cell death (apoptosis) under a broad range of physiologic conditions. Bcl-2 and several related proteins function to inhibit apoptosis, while other members of the Bcl-2 family, such as Bax and Bak, enhance cell death under various conditions. For instance, Bcl-x_L represses cell death, while its shorter form, Bcl-x_S, promotes apoptosis. Dimerization of another member of this family, Bad, with Bcl-x_L, results in displacement of Bax from Bcl-x_L/Bax complexes and restoration of Bax-mediated apoptosis. A Bcl-2-binding protein, designated Bag-1, lacks significant homology with Bcl-2 or with other Bcl-2-related proteins. Bag-1 appears to function to enhance Bcl-2 protection from cell death, suggesting that Bag-1 represents a new type of anti-cell death gene. This also suggests that certain routes of apoptosis induction, previously ascribed to Bcl-2-independent pathways, may instead reflect a requirement for a combination of Bcl-2 and Bag-1.

REFERENCES

1. Nunez, G., et al. 1990. Deregulated Bcl-2 gene expression selectively prolongs survival of growth factor-deprived hemopoietic cell lines. *J. Immunol.* 144: 3602-3610.
2. Hockenbery, D.M., et al. 1991. Bcl-2 protein is topographically restricted in tissues characterized by apoptotic cell death. *Proc. Natl. Acad. Sci. USA* 88: 6961-6965.
3. Oltvai, Z.N., et al. 1993. Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 74: 609-619.
4. Yin, X.M., et al. 1994. BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. *Nature* 369: 321-323.

CHROMOSOMAL LOCATION

Genetic locus: Bag1 (rat) mapping to 5q22.

PRODUCT

Bag-1 siRNA (r) 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 Bag-1 shRNA Plasmid (r): sc-61877-SH and Bag-1 shRNA (r) Lentiviral Particles: sc-61877-V as alternate gene silencing products.

For independent verification of Bag-1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-61877A, sc-61877B and sc-61877C.

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

Bag-1 siRNA (r) is recommended for the inhibition of Bag-1 expression in rat 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

Bag-1 (E-11): sc-376848 is recommended as a control antibody for monitoring of Bag-1 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 MarkerTM 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 Bag-1 gene expression knockdown using RT-PCR Primer: Bag-1 (r)-PR: sc-61877-PR (20 μ l, 524 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Gurusamy, N., et al. 2009. Cardioprotection by adaptation to ischaemia augments autophagy in association with Bag-1 protein. *J. Cell. Mol. Med.* 13: 373-387.

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.