

Bad siRNA (h): sc-29778

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. A protein designated Bad exhibits homology to Bcl-2, limited to the BH1 and BH2 domains. Bad functions to dimerize with Bcl-x_L and with Bcl-2, but not with Bax, Bcl-x_S, Mcl-1, A1 or itself. In mammalian cells, Bad binds with greater affinity to Bcl-x_L than to Bcl-2, and reverses the death repressor activity of Bcl-x_L but not Bcl-2. Dimerization of Bad with Bcl-x_L results in displacement of Bax from Bcl-x_L:Bax complexes, thereby causing restoration of Bax-mediated apoptosis.

CHROMOSOMAL LOCATION

Genetic locus: BAD (human) mapping to 11q13.1.

PRODUCT

Bad 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 Bad shRNA Plasmid (h): sc-29778-SH and Bad shRNA (h) Lentiviral Particles: sc-29778-V as alternate gene silencing products.

For independent verification of Bad (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-29778A, sc-29778B and sc-29778C.

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

Bad siRNA (h) is recommended for the inhibition of Bad 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

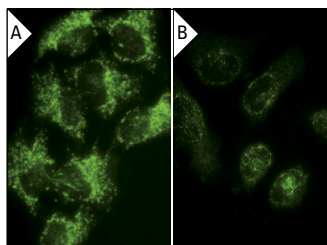
Bad (C-7): sc-8044 is recommended as a control antibody for Western Blotting (starting dilution 1:100, dilution range 1:100-1:1,000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) protein detection using the recommended secondary reagents listed below.

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 Bad gene expression knockdown using RT-PCR Primer: Bad (h)-PR: sc-29778-PR (20 μl, 520 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

DATA



Bad siRNA (h): sc-29778. Immunofluorescence staining of methanol-fixed, control HeLa (A) and Bad siRNA silenced HeLa (B) cells showing diminished cytoplasmic staining in the siRNA silenced cells. Cells probed with Bad (H-168): sc-7869.

SELECT PRODUCT CITATIONS

1. Panka, D.J., et al. 2006. The Raf inhibitor BAY 43-9006 (sorafenib) induces caspase-independent apoptosis in melanoma cells. *Cancer Res.* 66: 1611-1619.
2. Ogunwobi, O.O., et al. 2008. Statins inhibit proliferation and induce apoptosis in Barrett's esophageal adenocarcinoma cells. *Am. J. Gastroenterol.* 103: 825-837.
3. Liu, J.J., et al. 2015. 15,16-dihydrodanthronone I from the functional food *salvia miltiorrhiza* exhibits anticancer activity in human HL-60 leukemia cells: *in vitro* and *in vivo* studies. *Int. J. Mol. Sci.* 16: 19387-19400.
4. Cekanova, M., et al. 2015. Bcl-2 family protein, BAD is down-regulated in breast cancer and inhibits cell invasion. *Exp. Cell Res.* 331: 1-10.

RESEARCH USE

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