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Bax siRNA (h2): sc-44199

BACKGROUND

The Bcl-2 gene was isolated at the chromosomal breakpoint of t-bearing follicular B cell lymphomas. Bcl-2 blocks cell death following a variety of stimuli and confers a death-sparing effect to certain hematopoietic cell lines following growth factor withdrawal. Bcl-2 is localized to outer mitochondrial membranes and endoplasmic reticulum, as well as nuclear membranes. A related protein, designated Bax (Bcl-associated X protein), has extensive amino acid homology with Bcl-2 and both homodimerize and form heterodimers with Bcl-2. Overexpression of Bax accelerates apoptotic death induced by cytokine deprivation in an IL-3 dependent cell line. Bax also counters the death repressor activity of Bcl-2.

REFERENCES

1. Bakhshi, A., et al. 1985. Cloning the chromosomal breakpoint of t(14;18) human lymphomas: clustering around JH on chromosome 14 and near a transcriptional unit on 18. *Cell* 41: 899-906.
2. Vaux, D.L., et al. 1988. Bcl-2 promotes the survival of haemopoietic cells and cooperates with c-Myc to immortalize pre-B cells. *Nature* 335: 440-442.
3. Chen-Levy, Z., et al. 1989. The Bcl-2 candidate proto-oncogene product is a 24-kilodalton integral-membrane protein highly expressed in lymphoid cell lines and lymphomas carrying the t(14;18). *Mol. Cell. Biol.* 9: 701-710.
4. 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.
5. 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.

CHROMOSOMAL LOCATION

Genetic locus: BAX (human) mapping to 19q13.33.

PRODUCT

Bax siRNA (h2) 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 Bax shRNA Plasmid (h2): sc-44199-SH and Bax shRNA (h2) Lentiviral Particles: sc-44199-V as alternate gene silencing products.

For independent verification of Bax (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44199A, sc-44199B and sc-44199C.

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.

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

Bax siRNA (h2) is recommended for the inhibition of Bax 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 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

Bax (B-9): sc-7480 is recommended as a control antibody for monitoring of Bax 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 Marker™ 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 Bax gene expression knockdown using RT-PCR Primer: Bax (h2)-PR: sc-44199-PR (20 μ l, 494 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.