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Bcl-x_{S/L} shRNA (h2) Lentiviral Particles: sc-44246-V

BACKGROUND

The Bcl-2 gene was isolated at the chromosomal breakpoint of t(14;18) 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. A second protein, designated Bcl-associated X protein (Bax) p21, has extensive amino acid homology with Bcl-2 and both homodimerizes and heterodimerizes with Bcl-2. Over-expression of Bax accelerates apoptotic death induced by cytokine deprivation in an IL-3-dependent cell line, and Bax also counters the death repressor activity of Bcl-2. Bcl-x, one of several additional proteins with sequence homology to Bcl-2, is expressed as Bcl-x_L, a 233 amino acid protein with 43% sequence identity with Bcl-2 that suppresses cell death, and Bcl-x_S, a shorter variant that is 178 amino acids in length and lacks a 63 amino acid region (amino acids 126-188) found in Bcl-x_L and which functions as a dominant inhibitor of Bcl-2. A further apoptosis-inducing protein, Bad, dimerizes both with Bcl-x_L and to a lesser extent with Bcl-2, thus displacing Bax and inducing apoptosis.

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. Boise, L.H., et al. 1993. Bcl-x, a Bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. *Cell* 74: 597-608.
4. 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.
5. Sato, T., et al. 1994. Interactions among members of the Bcl-2 protein family analyzed with a yeast two-hybrid system. *Proc. Natl. Acad. Sci. USA* 91: 9238-9242.

CHROMOSOMAL LOCATION

Genetic locus: BCL2L1 (human) mapping to 20q11.21.

PRODUCT

Bcl-x_{S/L} shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Bcl-x_{S/L} siRNA (h2): sc-44246 and Bcl-x_{S/L} shRNA Plasmid (h2): sc-44246-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

Bcl-x_{S/L} shRNA (h2) Lentiviral Particles is recommended for the inhibition of Bcl-x_{S/L} expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Bcl-x_{S/L} (D-3): sc-271121 is recommended as a control antibody for monitoring of Bcl-x_{S/L} 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Bcl-x_{S/L} gene expression knockdown using RT-PCR Primer: Bcl-x_{S/L} (h2)-PR: sc-44246-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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