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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 



NBK shRNA (m) Lentiviral Particles: sc-45934-V

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 appears to function in several subcellular locations yet lacks any known motifs that would provide insight into its mechanism of action. A protein designated Bax p21 (for Bcl-associated X protein) has extensive amino acid homology with Bcl-2 and both hetero-dimerizes and homodimerizes with Bcl-2. Overexpression of Bax accelerates apoptotic death. Natural born killer (NBK), also known as Bik, is a protein that is functionally related to Bax, although the two proteins share very little sequence homology. NBK does not contain the conserved Bcl-2 homology domains (BH domains) characteristic of the Bcl-2 family. It does however, share nine amino acids with Bax in a region designated BH3, which may be the critical determinant for the NBK death-promoting activities.

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. 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.
3. 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.
4. Jacobson, M.D., et al. 1993. Bcl-2 blocks apoptosis in cells lacking mitochondrial DNA. *Nature* 361: 365-369.
5. Oltvai, Z.N., et al. 1993. Bcl-2 heterodimerizes *in vivo* with a conserved homology, bax, that accelerates programmed cell death. *Cell* 74: 609-619.

CHROMOSOMAL LOCATION

Genetic locus: Bik (mouse) mapping to 15 E1.

PRODUCT

NBK shRNA (m) 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 μ l frozen stock containing 1.0×10^6 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 NBK siRNA (m): sc-45934 and NBK shRNA Plasmid (m): sc-45934-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

NBK shRNA (m) Lentiviral Particles is recommended for the inhibition of NBK expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

NBK (E-20): sc-30552 is recommended as a control antibody for monitoring of NBK 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NBK gene expression knockdown using RT-PCR Primer: NBK (m)-PR: sc-45934-PR (20 μ l, 418 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.

PROTOCOLS

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