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Bmf shRNA (m) Lentiviral Particles: sc-45931-V

BACKGROUND

The BH3-only proteins, which include Blk, Bad, Bik, Hrk, Bid, Bim, NOXA, PUMA and Bmf, are proapoptotic members of the Bcl-2 family. Bcl-2 modifying factor (Bmf) is a BH3-only protein that binds prosurvival Bcl-2 family members to initiate apoptosis. Bmf is sequestered to Myosin V motors on Actin in the cytoskeleton by associating with Dynein light chain 2 (DLC2) homodimers. If the cell undergoes loss of attachment (anoikis), the cytoskeleton is disrupted and Bmf is released from DLC2. Bmf then translocates to the mitochondria, where Bcl-2 (an anti-apoptotic family member) is sequestered. The BH3 domain of Bmf facilitates binding to a hydrophobic groove on the surface of Bcl-2. Binding results in a caspase cascade leading to apoptosis. Bmf is widely expressed in tissues such as pancreas, liver and kidney, and in hematopoietic tissues. The gene encoding Bmf maps to chromosome 15q15.1.

REFERENCES

1. Bouillet, P., Metcalf, D., Huang, D.C., Tarlinton, D.M., Kay, T.W., Kontgen, F., Adams, J.M. and Strasser, A. 1999. Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science* 286: 1735-1738.
2. Huang, D.C. and Strasser, A. 2000. BH3-only proteins—essential initiators of apoptotic cell death. *Cell* 103: 839-842.
3. Naisbitt, S., Valtschanoff, J., Allison, D.W., Sala, C., Kim, E., Craig, A.M., Weinberg, R.J. and Sheng, M. 2000. Interaction of the postsynaptic density-95/guanylate kinase domain-associated protein complex with a light chain of Myosin V and Dynein. *J. Neurosci.* 20: 4524-4534.
4. Puthalakath, H., Villunger, A., O'Reilly, L.A., Beaumont, J.G., Coultas, L., Cheney, R.E., Huang, D.C. and Strasser, A. 2001. Bmf: a proapoptotic BH3-only protein regulated by interaction with the Myosin V Actin motor complex, activated by anoikis. *Science* 293: 1829-1832.

CHROMOSOMAL LOCATION

Genetic locus: Bmf (mouse) mapping to 2 E5.

PRODUCT

Bmf 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 Bmf siRNA (m): sc-45931 and Bmf shRNA Plasmid (m): sc-45931-SH as alternate gene silencing products.

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.

APPLICATIONS

Bmf shRNA (m) Lentiviral Particles is recommended for the inhibition of Bmf 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

Bmf (C-20): sc-20183 is recommended as a control antibody for monitoring of Bmf 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 Bmf gene expression knockdown using RT-PCR Primer: Bmf (m)-PR: sc-45931-PR (20 μ l). 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.

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.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.