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# AIF shRNA (h2) Lentiviral Particles: sc-44243-V

## BACKGROUND

A key event in the apoptotic process is the opening of the mitochondrial permeability transition pore, an event that is regulated by Bcl-2 family proteins, resulting in the release of several proteins from the mitochondrial intermembrane space. Several of these proteins participate in apoptosis, including cytochrome c, procaspases 2, 3, and 9, and AIF (apoptosis-inducing factor). AIF has been shown to cause DNA fragmentation and chromatin condensation and to induce the release of cytochrome c and caspase-9 from mitochondria. Bcl-2 overexpression has been shown to prevent the release of AIF from mitochondria, but not to block its apoptogenic activity.

## REFERENCES

1. Susin, S.A., et al. 1996. Bcl-2 inhibits the mitochondrial release of an apoptogenic protease. *J. Exp. Med.* 184: 1331-1341.
2. Zamzami, N., et al. 1996. Mitochondrial control of nuclear apoptosis. *J. Exp. Med.* 183: 1533-1544.
3. Kluck, R.M., et al. 1997. The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science* 275: 1132-1136.
4. Green, D.R., et al. 1998. Mitochondria and apoptosis. *Science* 281: 1309-1312.
5. Mancini, M., et al. 1998. The caspase-3 precursor has a cytosolic and mitochondrial distribution: implications for apoptotic signaling. *J. Cell Biol.* 140: 1485-1495.
6. Susin, S.A., et al. 1999. Mitochondrial release of caspase-2 and -9 during the apoptotic process. *J. Exp. Med.* 189: 381-394.
7. Susin, S.A., et al. 1999. Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 397: 441-446.

## CHROMOSOMAL LOCATION

Genetic locus: AIFM1 (human) mapping to Xq26.1.

## PRODUCT

AIF 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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 AIF siRNA (h2): sc-44243 and AIF shRNA Plasmid (h2): sc-44243-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

AIF shRNA (h2) Lentiviral Particles is recommended for the inhibition of AIF expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

AIF (E-11): sc-390619 is recommended as a control antibody for monitoring of 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 IgM-HRP: sc-2064 (dilution range: 1:500-1:5,000), TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgM-FITC: sc-2082 (dilution range: 1:100-1:400) or goat anti-mouse IgM-TR: sc-2983 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor AIF gene expression knockdown using RT-PCR Primer: AIF (h2)-PR: sc-44243-PR (20  $\mu$ l, 536 bp). 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](http://www.scbt.com) or our catalog for detailed protocols and support products.