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AIF siRNA (h2): sc-44243

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 was shown to cause DNA fragmentation and chromatin condensation, and to induce the release of cytochrome c and caspase-9 from mitochondria. Bcl-2 over-expression was 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.
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CHROMOSOMAL LOCATION

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

PRODUCT

AIF 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 AIF shRNA Plasmid (h2): sc-44243-SH and AIF shRNA (h2) Lentiviral Particles: sc-44243-V as alternate gene silencing products.

For independent verification of AIF (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-44243A, sc-44243B and sc-44243C.

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

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

AIF (E-1): sc-13116 is recommended as a control antibody for monitoring of AIF 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 AIF gene expression knockdown using RT-PCR Primer: AIF (h2)-PR: sc-44243-PR (20 μ l, 536 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.