SANTA CRUZ BIOTECHNOLOGY, INC.

cytochrome c-t siRNA (m): sc-108096

BACKGROUND

Cytochrome c is a well characterized mobile electron transport protein that is essential to energy conversion in all aerobic organisms. In mammalian cells, this highly conserved protein is normally localized to the mitochondrial intermembrane space. More recent studies have identifed cytosolic cytochrome c as a factor necessary for activation of apoptosis. During apoptosis, cytochrome c is translocated from the mitochondrial membrane to the cytosol, where it is required for activation of caspase-3 (CPP32). Overexpression of Bcl-2 has been shown to prevent the translocation of cytochrome c, thereby blocking the apoptotic process. Overexpression of Bax has been shown to induce the release of cytochrome c and to induce cell death. The release of cytochrome c from the mitochondria is thought to trigger an apoptotic cascade, whereby Apaf-1 binds to Apaf-3 (caspase-9) in a cytochrome c-dependent manner, leading to caspase-9 cleavage of caspase-3.

REFERENCES

1. Gonzales, D.H., et al. 1990. Biogenesis of mitochrondrial c-type cytochromes. J. Bioenerg. Biomembr. 22: 753-768.
2. Lehninger, A.L., et al. 1993. Principles of Biochemistry, 2nd ed. New York: Worth Publishers, Inc., 480-483.
3. Liu, X., et al. 1996. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell 86: 147-157.
4. Yang, J., et al. 1997. Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. Science 275: 1129-1132.
5. 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.
6. Zou, H., et al. 1997. Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 90: 405-413.
7. Li, P., et al. 1997. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91: 479-489.
8. Rosse, T., et al. 1998. Bcl-2 prolongs cell survival after Bax-induced release of cytochrome c. Nature 391: 496-499.

CHROMOSOMAL LOCATION

Genetic locus: Cyct (mouse) mapping to 2 C3.

PRODUCT

cytochrome c-t siRNA (m) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see cytochrome c-t shRNA Plasmid (m): sc-108096-SH and cytochrome c-t shRNA (m) Lentiviral Particles: sc-108096-V as alternate gene silencing products.

For independent verification of cytochrome c-t (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-108096A, sc-108096B and sc-108096C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

cytochrome c-t siRNA (m) is recommended for the inhibition of cytochrome c-t expression in mouse 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 µM in 66 µl. 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

cytochrome c (A-8): sc-13156 is recommended as a control antibody for monitoring of cytochrome c-t 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 cytochrome c-t gene expression knockdown using RT-PCR Primer: cytochrome c-t (m)-PR: sc-108096-PR (20 µl). 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.

PROTOCOLS

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