

BCLAF1 siRNA (h): sc-72633

BACKGROUND

Apoptosis defines a set of cascades which, when initiated, programs the cell to undergo lethal changes such as membrane blebbing, mitochondrial breakdown and DNA fragmentation. Bcl-2 is one of many key regulators of apoptosis which are essential for proper development, tissue homeostasis and protection against foreign pathogens. BCLAF1 (Bcl-2-associated transcription factor 1), also known as BTF, is a 920 amino acid protein that localizes to both the nucleus and the cytoplasm. Expressed throughout the body, BCLAF1 functions as a death-promoting factor that interacts with and represses the transcription of Bcl-2, thereby influencing the regulation of apoptosis. Over-expression of BCLAF1 results in the relocation of BCLAF1 to the nuclear envelope and the subsequent induction of apoptosis, an event that may occur as a result of DNA damage. Four isoforms of BCLAF1 exist due to alternative splicing events.

REFERENCES

1. Kasof, G.M., et al. 1999. BTF, a novel death-promoting transcriptional repressor that interacts with Bcl-2-related proteins. *Mol. Cell. Biol.* 19: 4390-4404.
2. Tai, H.H., et al. 2003. CHD1 associates with NCoR and histone deacetylase as well as with RNA splicing proteins. *Biochem. Biophys. Res. Commun.* 308: 170-176.
3. Haraguchi, T., et al. 2004. Emerin binding to BTF, a death-promoting transcriptional repressor, is disrupted by a missense mutation that causes Emery-Dreifuss muscular dystrophy. *Eur. J. Biochem.* 271: 1035-1045.
4. Beausoleil, S.A., et al. 2004. Large-scale characterization of HeLa cell nuclear phosphoproteins. *Proc. Natl. Acad. Sci. USA* 101: 12130-12135.
5. Mansharamani, M. and Wilson, K.L. 2005. Direct binding of nuclear membrane protein MAN1 to emerin *in vitro* and two modes of binding to barrier-to-autointegration factor. *J. Biol. Chem.* 280: 13863-13870.
6. Nousiainen, M., et al. 2006. Phosphoproteome analysis of the human mitotic spindle. *Proc. Natl. Acad. Sci. USA* 103: 5391-5396.

CHROMOSOMAL LOCATION

Genetic locus: BCLAF1 (human) mapping to 6q23.3.

PRODUCT

BCLAF1 siRNA (h) 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 BCLAF1 shRNA Plasmid (h): sc-72633-SH and BCLAF1 shRNA (h) Lentiviral Particles: sc-72633-V as alternate gene silencing products.

For independent verification of BCLAF1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-72633A, sc-72633B and sc-72633C.

RESEARCH USE

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

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

BCLAF1 siRNA (h) is recommended for the inhibition of BCLAF1 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

BCLAF1 (M33-P5B11): sc-101388 is recommended as a control antibody for monitoring of BCLAF1 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 BCLAF1 gene expression knockdown using RT-PCR Primer: BCLAF1 (h)-PR: sc-72633-PR (20 μ l, 513 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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