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Mcl-1_L shRNA (h) Lentiviral Particles: sc-43912-V

BACKGROUND

B cell CLL/lymphoma 2 (Bcl-2) blocks cell death following a variety of stimuli and confers a death-sparing effect to certain hematopoietic cell lines following growth factor withdrawal. Myeloid cell leukemia 1 (Mcl-1) shares sequence homology with Bcl-2 and further resembles Bcl-2 in that its expression promotes cell viability. p53 and Mcl-1 demonstrate opposing effects on mitochondrial apoptosis by mediating Bcl-2 antagonist killer (Bak) activity. Mcl-1 is an important and specific regulator that is necessary for the homeostasis of early hematopoietic progenitors. Glycogen synthase kinase 3 (GSK-3) controls Mcl-1 stability, which has an effect on the regulation of apoptosis by growth factors, PI 3-kinase and Akt. Mice with a deficiency of the Mcl-1 protein show a significant reduction in B and T lymphocytes similar to the effects observed in IL-7- or IL-7R-deficient mice. The Mcl-1 mRNA is alternatively spliced into a long and a short form of the protein, designated Mcl-1_L and Mcl-1_S, respectively. Mcl-1_S, unlike Mcl-1_L, does not interact with proapoptotic Bcl-2-related proteins.

REFERENCES

1. Kozopas, K.M., et al. 1993. Mcl-1, a gene expressed in programmed myeloid cell differentiation, has sequence similarity to Bcl-2. *Proc. Natl. Acad. Sci. USA* 90: 3516-3520.
2. Craig, R.W., et al. 1994. Human and mouse chromosomal mapping of the myeloid cell leukemia-1 gene: Mcl-1 maps to human chromosome 1q21, a region that is frequently altered in preneoplastic and neoplastic disease. *Genomics* 23: 457-463.
3. Rinkenberger, J.L., et al. 2000. Mcl-1 deficiency results in peri-implantation embryonic lethality. *Genes Dev.* 14: 23-27.
4. Opferman, J.T., et al. 2003. Development and maintenance of B and T lymphocytes requires antiapoptotic Mcl-1. *Nature* 426: 671-676.

CHROMOSOMAL LOCATION

Genetic locus: MCL1 (human) mapping to 1q21.3.

PRODUCT

Mcl-1_L shRNA (h) 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 µl frozen stock containing 1.0 x 10⁶ 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 Mcl-1_L siRNA (h): sc-43912 and Mcl-1_L shRNA Plasmid (h): sc-43912-SH as alternate gene silencing products.

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.

APPLICATIONS

Mcl-1L shRNA (h) Lentiviral Particles is recommended for the inhibition of Mcl-1L expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

Mcl-1_L (K-20): sc-958 is recommended as a control antibody for monitoring of Mcl-1_L 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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 goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Mcl-1_L gene expression knockdown using RT-PCR Primer: Mcl-1_L (h)-PR: sc-43912-PR (20 µl, 455 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.

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.

PROTOCOLS

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