

Mel-18 siRNA (m): sc-38192

BACKGROUND

In *Drosophila*, Polycomb (PcG) gene family encodes chromatin proteins that are required for the repression of homeotic loci in embryonic development. Mel-18 and Bmi-1 are mammalian homologs of *Drosophila* PcG group proteins, as they are similarly expressed during development and implicated in the regulation of gene expression, axial skeleton development, and the control of proliferation and survival of hematopoietic cells. Mel-18 directly binds to DNA through a ring-finger motif and preferentially associates with juxtaposed enhancer elements on various genes, including Bcl-2, c-myc and Hox. Mel-18 is an immediate early response gene within the c-Myc/Cdc25 signaling cascade that exhibits tumor suppressor activity and negatively regulates cell cycle progression by blocking S phase entry. Alternatively, Bmi-1 has been identified as a potent oncogene as it contributes to the transcriptional activation of genes implicated in early lymphoid development. Proviral activation of Bmi-1 expression corresponds to enhanced gene-specific activation of other proto-oncogenes, including c-Myc and pim, subsequently resulting in the progression of lymphomagenesis.

REFERENCES

1. Tagawa, M., et al. 1990. Expression of novel DNA-binding protein with zinc finger structure in various tumor cells. *J. Biol. Chem.* 265: 20021-20026.
2. Goebel, M.G. 1991. The Bmi-1 and Mel-18 gene products define a new family of DNA-binding proteins involved in cell proliferation and tumorigenesis. *Cell* 66: 623.
3. van Lohuizen, M., et al. 1991. Sequence similarity between the mammalian Bmi-1 proto-oncogene and the *Drosophila* regulatory genes Psc and Suz2. *Nature* 353: 353-355.
4. Ishida, A., et al. 1993. Cloning and chromosome mapping of the human Mel-18 gene which encodes a DNA-binding protein with a new "RING-finger" motif. *Gene* 129: 249-255.
5. Kanno, M., et al. 1995. Mel-18, a Polycomb group-related mammalian gene, encodes a transcriptional negative regulator with tumor suppressive activity. *EMBO J.* 14: 5672-5678.
6. Akasaka, T., et al. 1996. A role for Mel-18, a Polycomb group-related vertebrate gene, during the anteroposterior specification of the axial skeleton. *Development* 122: 1513-1522.

CHROMOSOMAL LOCATION

Genetic locus: Pcgf2 (mouse) mapping to 11 D.

PRODUCT

Mel-18 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Mel-18 shRNA Plasmid (m): sc-38192-SH and Mel-18 shRNA (m) Lentiviral Particles: sc-38192-V as alternate gene silencing products.

For independent verification of Mel-18 (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-38192A, sc-38192B and sc-38192C.

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

Mel-18 siRNA (m) is recommended for the inhibition of Mel-18 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 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

Mel-18 (H-1): sc-515329 is recommended as a control antibody for monitoring of Mel-18 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 MarkerTM 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 Mel-18 gene expression knockdown using RT-PCR Primer: Mel-18 (m)-PR: sc-38192-PR (20 μ l, 577 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.

PROTOCOLS

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