

Mel-18 siRNA (h): sc-38191

BACKGROUND

In *Drosophila*, Polycomb (Pc-g) gene family encodes chromatin proteins that are required for the repression of homeotic loci in embryonic development. Mel-18 and Bmi-1, mammalian homologs of *Drosophila* Pc-g group proteins, are similarly expressed during development and implicated in the regulation of gene expression, axial skeleton development, control of proliferation and survival of haematopoietic 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 Su(z)2. *Nature* 353: 353-355.

CHROMOSOMAL LOCATION

Genetic locus: PCGF2 (human) mapping to 17q12.

PRODUCT

Mel-18 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 Mel-18 shRNA Plasmid (h): sc-38191-SH and Mel-18 shRNA (h) Lentiviral Particles: sc-38191-V as alternate gene silencing products.

For independent verification of Mel-18 (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-38191A, sc-38191B and sc-38191C.

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 (h) is recommended for the inhibition of Mel-18 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

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 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 Mel-18 gene expression knockdown using RT-PCR Primer: Mel-18 (h)-PR: sc-38191-PR (20 μ l, 560 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Mitra, S., et al. 2018. *Ehrlichia chaffeensis* TRP120 effector targets and recruits host polycomb group proteins for degradation to promote intracellular infection. *Infect. Immun.* 86 pii: e00845-17.

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.