

MIA siRNA (h): sc-40742

BACKGROUND

Tumorigenesis is a process that is mediated by a network of membrane, cytosolic and nuclear associated factors, which regulate proliferation and cell-matrix interaction through signaling cascades. The phenotype of malignant melanomas *in vivo* depends on the global expression of stimulatory or inhibitory factors generated in both the tumors cells and their environment. One example, Melanoma inhibitory activity (cartilage-derived retinoic acid-sensitive protein (CD-RAP), MIA) is a Src homology 3 (SH3)-like domain containing protein that is secreted from chondrocytes and malignant melanoma cells. MIA is translated as a 131-amino acid pro-form and processed into a mature 107-amino acid protein after cleavage of a secretion signal. MIA is expressed during chondrogenesis and in mature chondrocytes, suggesting that MIA is necessary for normal cartilage cell phenotype. MIA mRNA is present in carcinomas of the colon, ovary, kidney and head/neck, and may represent a marker to monitor melanomic activity.

REFERENCES

1. Blesch, A., et al. 1994. Cloning of a novel malignant melanoma-derived growth-regulatory protein, MIA. *Cancer Res.* 54: 5695-5701.
2. Bosserhoff, A.K., et al. 1997. Mouse CD-RAP/MIA gene: structure, chromosomal localization and expression in cartilage and chondrosarcoma. *Dev. Dyn.* 208: 516-525.
3. Perez, R.P., et al. 2000. Expression of melanoma inhibitory activity in melanoma and nonmelanoma tissue specimens. *Hum. Pathol.* 31: 1381-1388.
4. Loughheed, J.C., et al. 2001. Structure of melanoma inhibitory activity protein, a member of a recently identified family of secreted proteins. *Proc. Natl. Acad. Sci. USA* 98: 5515-5520.
5. Stoll, R., et al. 2001. The extracellular human melanoma inhibitory activity (MIA) protein adopts an SH3 domain-like fold. *EMBO J.* 20: 340-349.

CHROMOSOMAL LOCATION

Genetic locus: MIA (human) mapping to 19q13.2.

PRODUCT

MIA 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 MIA shRNA Plasmid (h): sc-40742-SH and MIA shRNA (h) Lentiviral Particles: sc-40742-V as alternate gene silencing products.

For independent verification of MIA (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-40742A, sc-40742B and sc-40742C.

PROTOCOLS

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

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

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

MIA (C-10): sc-377375 is recommended as a control antibody for monitoring of MIA 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 MIA gene expression knockdown using RT-PCR Primer: MIA (h)-PR: sc-40742-PR (20 μ l). 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.