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MRTF-A shRNA (h) Lentiviral Particles: sc-43944-V

BACKGROUND

Serum response factor (SRF) is a transcription factor that binds the serum response element (SRE), a sequence that mediates the transient response of many cellular genes to growth stimulation. SRF regulates the transient response of several muscle genes in response to growth factors and recruits accessory myogenic factors to activate these muscle genes. SRF is required for the formation of vertebrate mesoderm leading to the origin of the cardiovascular system. Myocardin, in association with SRF in cardiac muscle cells, activates cardiac muscle promoters. Myocardin-related transcription factor A (MRTF-A), also known as MKL1, interacts with SRF and stimulates its transcriptional activity.

REFERENCES

1. Norman, C., et al. 1988. Isolation and properties of cDNA clones encoding SRF, a transcription factor that binds to the c-fos serum response element. *Cell* 55: 989-1003.
2. Treisman, R. 1990. The SRE: a growth factor responsive transcriptional regulator. *Semin. Cancer Biol.* 1: 47-58.
3. Hill, C.S., et al. 1993. Functional analysis of a growth factor-responsive transcription factor complex. *Cell* 73: 395-406.
4. Wang, D.Z., et al. 2002. Potentiation of serum response factor activity by a family of Myocardin-related transcription factors. *Proc. Natl. Acad. Sci. USA* 99: 14855-14860.
5. Lockman, K., et al. 2004. Sphingosine 1-phosphate stimulates smooth muscle cell differentiation and proliferation by activating separate serum response factor co-factors. *J. Biol. Chem.* 279: 42422-42430.
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CHROMOSOMAL LOCATION

Genetic locus: MKL1 (human) mapping to 22q13.1.

PRODUCT

MRTF-A shRNA (h) Lentiviral Particles are concentrated, transduction-ready viral particles containing a target-specific construct that encodes a 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 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 MRTF-A siRNA (h): sc-43944 and MRTF-A shRNA Plasmid (h): sc-43944-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

MRTF-A shRNA (h) Lentiviral Particles is recommended for the inhibition of MRTF-A expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

MRTF-A (G-7): sc-398675 is recommended as a control antibody for monitoring of MRTF-A 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-mouse IgM-HRP: sc-2064 (dilution range: 1:500-1:5,000), TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgM-FITC: sc-2082 (dilution range: 1:100-1:400) or goat anti-mouse IgM-TR: sc-2983 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor MRTF-A gene expression knockdown using RT-PCR Primer: MRTF-A (h)-PR: sc-43944-PR (20 μ l, 560 bp). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ 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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