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Myocardin shRNA (m) Lentiviral Particles: sc-43954-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-binding sites are also constitutive promoter elements in many muscle-specific promoters. Myocardin associates with SRF in cardiac muscle cells to activate cardiac muscle promoters. Myocardin is also expressed in smooth muscle cells and appears to play a role in cell differentiation. Specifically, myocardin is expressed in vascular smooth muscle within the aortic arteries and pulmonary outflow tract as well as in the genitourinary tract and gastrointestinal tract. Myocardin is absent in the coronary vasculature, dorsal aorta, skeletal muscle or other non-muscle tissue types. Myocardin belongs to the SAP (SAF-A/B, Acinus and PIAS) domain family of nuclear proteins which includes hnRNP U and PIAS. The SAP domain may play a role in targeting proteins to specific chromosomal locations.

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. Boxer, L.M., et al. 1989. The sarcomeric Actin CArG-binding factor is indistinguishable from the c-Fos serum response factor. *Mol. Cell. Biol.* 9: 515-522.
3. Treisman, R. 1990. The SRE: a growth factor responsive transcriptional regulator. *Sem. Cancer Biol.* 1: 47-58.
4. Hill, C.S., et al. 1993. Functional analysis of a growth factor-responsive transcription factor complex. *Cell* 73: 395-406.
5. Aravind, L. and Koonin, E.V. 2000. SAP — a putative DNA-binding motif involved in chromosomal organization. *Trends Biochem. Sci.* 25: 112-114.
6. Wang, D., et al. 2001. Activation of cardiac gene expression by Myocardin, a transcriptional cofactor for serum response factor. *Cell* 105: 851-862.

CHROMOSOMAL LOCATION

Genetic locus: Myocd (mouse) mapping to 11 B3.

PRODUCT

Myocardin shRNA (m) 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 Myocardin siRNA (m): sc-43954 and Myocardin shRNA Plasmid (m): sc-43954-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

Myocardin shRNA (m) Lentiviral Particles is recommended for the inhibition of Myocardin expression in mouse 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

Myocardin (H-300): sc-33766 is recommended as a control antibody for monitoring of Myocardin 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 Myocardin gene expression knockdown using RT-PCR Primer: Myocardin (m)-PR: sc-43954-PR (20 μ l, 598 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.

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