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# Myf-6 shRNA (m) Lentiviral Particles: sc-43522-V

## BACKGROUND

Differentiation of myogenic cells is regulated by multiple positively and negatively acting factors. One well characterized family of helix-loop-helix (HLH) proteins known to play an important role in the regulation of muscle cell development includes MyoD, myogenin, Myf-5 and Myf-6 (also designated MRF-4 or herculin). Most muscle cells express either MyoD or Myf-5 in the committed state, but when induced to differentiate, all turn on expression of myogenin. MyoD transcription factors form heterodimers with products of a more widely expressed family of bHLH genes, the E family, which consists of at least three distinct genes: E2A, IF2 and HEB. MyoD-E heterodimers bind avidly to consensus (CANNTG) E box target sites that are functionally important elements in the upstream regulatory sequences of many muscle-specific terminal differentiation genes.

## REFERENCES

1. Braun, T., et al. 1989. A novel human muscle factor related to but distinct from MyoD1 induces myogenic conversion in 10T1/2 fibroblasts. *EMBO J.* 8: 701-709.
2. Rhodes, S.J. et al. 1989. Identification of MRF4: a new member of the muscle regulatory factor gene family. *Genes Dev.* 3: 2050-2061.
3. Wright, W.E., et al. 1989. Myogenin, a factor regulating myogenesis, has a domain homologous to MyoD. *Cell* 56: 607-617.
4. Miner, J.H. et al. 1990. Herculins, a fourth member of the MyoD family of myogenic regulatory genes. *Proc. Natl. Acad. Sci. USA* 87: 1089-1093.
5. Braun, T., et al. 1990. Myf-6, a new member of the human gene family of myogenic determination factors: evidence for a gene cluster on chromosome 12. *EMBO J.* 9: 821-831.
6. Hollenberg, S.M., et al. 1993. Use of a conditional Myo D transcription factor in studies of Myo D trans-activation and muscle determination. *Proc. Natl. Acad. Sci. USA* 90: 8028-8032.

## CHROMOSOMAL LOCATION

Genetic locus: Myf6 (mouse) mapping to 10 D1.

## PRODUCT

Myf-6 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 Myf-6 siRNA (m): sc-43522 and Myf-6 shRNA Plasmid (m): sc-43522-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ\text{C}$ . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ\text{C}$  for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

Myf-6 shRNA (m) Lentiviral Particles is recommended for the inhibition of Myf-6 expression in mouse cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

Myf-6 (242): sc-784 is recommended as a control antibody for monitoring of Myf-6 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 Myf-6 gene expression knockdown using RT-PCR Primer: Myf-6 (m)-PR: sc-43522-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ\text{C}$  and the extension temperature should be  $68-72^\circ\text{C}$ .

## 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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