

Myf-5 siRNA (h): sc-35988

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 include Myo D, myogenin, Myf-5 and Myf-6 (also designated MRF-4 or herculin). Of interest, most muscle cells express either Myo D or Myf-5 in the committed state, but when induced to differentiate, all turn on expression of myogenin. Myo D 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. Myo D-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 Myo D1 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 Myo D. *Cell* 56: 607-617.
4. Miner, J.H., et al. 1990. Herculin, a fourth member of the Myo D 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. Thayer, M.J., et al. 1993. A cellular factor stimulates the DNA-binding activity of Myo D and E47. *Proc. Natl. Acad. Sci. USA* 90: 6483-6487.
7. 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.
8. Neuhold, L.A., et al. 1993. HLH forced dimers: tethering Myo D to E47 generates a dominant positive myogenic factor insulated from negative regulation by Id. *Cell* 74: 1033-1042.

CHROMOSOMAL LOCATION

Genetic locus: MYF5 (human) mapping to 12q21.31.

PRODUCT

Myf-5 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 Myf-5 shRNA Plasmid (h): sc-35988-SH and Myf-5 shRNA (h) Lentiviral Particles: sc-35988-V as alternate gene silencing products.

For independent verification of Myf-5 (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-35988A, sc-35988B and sc-35988C.

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

Myf-5 siRNA (h) is recommended for the inhibition of Myf-5 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

Myf-5 (B-2): sc-518039 is recommended as a control antibody for monitoring of Myf-5 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 Myf-5 gene expression knockdown using RT-PCR Primer: Myf-5 (h)-PR: sc-35988-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.

PROTOCOLS

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