

MyoD siRNA (r): sc-270217

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). Of interest, 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, Irf-2 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

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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.
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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. Thayer, M.J., et al. 1993. A cellular factor stimulates the DNA-binding activity of MyoD and E47. *Proc. Natl. Acad. Sci. USA* 90: 6483-6487.
7. Hollenberg, S.M., et al. 1993. Use of a conditional MyoD transcription factor in studies of MyoD *trans*-activation and muscle determination. *Proc. Natl. Acad. Sci. USA* 90: 8028-8032.
8. Neuhold, L.A., et al. 1993. HLH forced dimers: tethering MyoD to E47 generates a dominant positive myogenic factor insulated from negative regulation by Id. *Cell* 74: 1033-1042.

CHROMOSOMAL LOCATION

Genetic locus: MyoD1 (rat) mapping to 1q22.

PRODUCT

MyoD siRNA (r) 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 MyoD shRNA Plasmid (r): sc-270217-SH and MyoD shRNA (r) Lentiviral Particles: sc-270217-V as alternate gene silencing products.

For independent verification of MyoD (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270217A, sc-270217B and sc-270217C.

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

MyoD siRNA (r) is recommended for the inhibition of MyoD expression in rat 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

MyoD (G-1): sc-377460 is recommended as a control antibody for monitoring of MyoD 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 MyoD gene expression knockdown using RT-PCR Primer: MyoD (r)-PR: sc-270217-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.