SANTA CRUZ BIOTECHNOLOGY, INC.

MyoD siRNA (m): sc-35991

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, 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.

CHROMOSOMAL LOCATION

Genetic locus: Myod1 (mouse) mapping to 7 B4.

PRODUCT

MyoD siRNA (m) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MyoD shRNA Plasmid (m): sc-35991-SH and MyoD shRNA (m) Lentiviral Particles: sc-35991-V as alternate gene silencing products.

For independent verification of MyoD (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35991A, sc-35991B and sc-35991C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

MyoD siRNA (m) is recommended for the inhibition of MyoD expression in mouse 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 µM in 66 µl. 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 (m)-PR: sc-35991-PR (20 µl, 530 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Wang, H., et al. 2007. NFκB regulation of YY1 inhibits skeletal myogenesis through transcriptional silencing of myofibrillar genes. Mol. Cell. Biol. 27: 4374-4387.
2. Hewitt, J., et al. 2008. The muscle transcription factor MyoD promotes osteoblast differentiation by stimulation of the osterix promoter. Endocrinology 149: 3698-3707.
3. Hirai, H., et al. 2010. MyoD regulates apoptosis of myoblasts through microRNA-mediated down-regulation of Pax3. J. Cell Biol. 191: 347-365.
4. Eom, G.H., et al. 2011. Histone methyltransferase SETD3 regulates muscle differentiation. J. Biol. Chem. 286: 34733-34742.
5. Kim, J.W., et al. 2011. Tip60 regulates myoblast differentiation by enhancing the transcriptional activity of MyoD via their physical interactions. FEBS J. 278: 4394-4404.
6. Mohamed, J.S., et al. 2013. Ankyrin repeat domain protein 2 and inhibitor of DNA binding 3 cooperatively inhibit myoblast differentiation by physical interaction. J. Biol. Chem. 288: 24560-24568.

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.