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Diagnostik & molekulare Diagnostik



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CUG-BP2 siRNA (m): sc-44555



The Power to Question

BACKGROUND

Myotonic dystrophy (DM) is an autosomal dominant neuromuscular disease that is associated with a (CTG)n repeat expansion in the 3'-untranslated region of the myotonin protein kinase gene (DMPK). CUG-BP1 and CUG-BP2 are proteins that bind specifically to (CUG)8 oligonucleotides in vitro. While CUG-BP1 has the major binding activity in normal cells, nuclear CUG-BP2 binding activity increases in DM cells. Both CUG-BP1 and CUG-BP2 are isoforms of a novel heterogeneous nuclear ribonucleoprotein (hnRNP), hNab50. CUG-BP1, an RNA CUG triplet repeat binding protein, regulates splicing and translation of various RNAs. Expansion of RNA CUG repeats in the DMPK in DM is associated with alterations in binding activity of CUG-BP1 as well as alterations in the translation of the C/EBPB transcription factor. CUG-BP1 is an important regulator of initiation from different AUG codons of C/EBPB mRNA. In normal cells, CUG-BP1 up-regulates the p21 protein during differentiation by inducing the translation of p21 via binding to a GC-rich sequence located within the 5' region of p21 mRNA. In DM cells, failure to accumulate CUG-BP1 leads to a reduction of p21 and alterations in other proteins responsible for cell cycle withdrawl.

REFERENCES

- Timchenko, L.T., et al. 1996. Identification of a (CUG)n triplet repeat RNAbinding protein and its expression in myotonic dystrophy. Nucleic Acids Res. 24: 4407-4414.
- 2. Timchenko, N.A., et al. 1999. CUG repeat binding protein (CUG-BP1) interacts with the 5'-region of C/EBP β mRNA and regulates translation of C/EBP β isoforms. Nucleic Acids. Res. 27: 4517-4525.
- Takahashi, N., et al. 2000. The CUG-binding protein binds specifically to UG dinucleotide repeats in yeast three-hybrid system. Biochem. Biophys. Res. Commun. 277: 518-523.
- Timchenko, N.A., et al. 2001. RNA CUG repeats sequester CUG-BP1 and alter protein levels and activity of CUG-BP1. J. Biol. Chem. 276: 7820-7826.
- 5. Timchenko, N.A., et al. 2001. Molecular basis for imparied muscle differentiation in myotonic dystrophy. Mol. Cell. Biol. 21: 6927-6938.

CHROMOSOMAL LOCATION

Genetic locus: Celf2 (mouse) mapping to 2 A1.

PRODUCT

CUG-BP2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CUG-BP2 shRNA Plasmid (m): sc-44555-SH and CUG-BP2 shRNA (m) Lentiviral Particles: sc-44555-V as alternate gene silencing products.

For independent verification of CUG-BP2 (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-44555A, sc-44555B and sc-44555C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

CUG-BP2 siRNA (m) is recommended for the inhibition of CUG-BP2 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

CUG-BP2 (1H2): sc-47731 is recommended as a control antibody for monitoring of CUG-BP2 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-lgG κ BP-HRP: sc-516102 or m-lgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz MarkerTM Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-lgG κ BP-FITC: sc-516140 or m-lgG 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 CUG-BP2 gene expression knockdown using RT-PCR Primer: CUG-BP2 (m)-PR: sc-44555-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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

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