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CUG-BP2 shRNA (h) Lentiviral Particles: sc-44554-V

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/EBP β transcription factor. CUG-BP1 is an important regulator of initiation from different AUG codons of C/EBP β 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 withdrawal.

REFERENCES

1. Timchenko, L.T., et al. 1996. Identification of a (CUG)_n triplet repeat RNA-binding 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.
3. 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.

CHROMOSOMAL LOCATION

Genetic locus: CUGBP2 (human) mapping to 10p14.

PRODUCT

CUG-BP2 shRNA (h) 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 μ l frozen stock containing 1.0×10^6 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 CUG-BP2 siRNA (h): sc-44554 and CUG-BP2 shRNA Plasmid (h): sc-44554-SH as alternate gene silencing products.

STORAGE

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

PROTOCOLS

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

APPLICATIONS

CUG-BP2 shRNA (h) Lentiviral Particles is recommended for the inhibition of CUG-BP2 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

CUG-BP2 (N-15): sc-27087 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CUG-BP2 gene expression knockdown using RT-PCR Primer: CUG-BP2 (h)-PR: sc-44554-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.