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SANTA CRUZ BIOTECHNOLOGY, INC.

PABPN1 shRNA (h) Lentiviral Particles: sc-44819-V

BACKGROUND

Oculopharyngeal muscular dystrophy (OPMD), an autosomal dominant late-onset progressive disease, generally presents in patients 50-70 years of age with dysphagia, ptosis and proximal limb weakness. OPMD is caused by the abnormal expansion of a $(GCG)_n$ trinucleotide repeat in the coding region of the polyadenylate binding protein nuclear 1 (PABPN1, also designated PABP2) gene. In the wildtype form of PABPN1, $(GCG)_6$ codes for the first six alanines in a homopolymeric stretch of ten alanines. In most individuals with OPMD, this $(GCG)_6$ repeat is expanded to $(GCG)_{8\text{-}13}$, leading to a stretch of 12-17 alanines in mutant PABPN1. Mutated PABPN1 forms aggregates consisting of tubular filaments within the nuclei of skeletal muscle fibers. The PABPN1 protein contains two RNA binding domains, a ribonucleoprotein-type RNA binding domain (RNP domain) and an arginine-rich C-terminal domain, which promotes self-association of PABPN1 and cooperative binding to RNA.

REFERENCES

1. Scheuermann, T., et al. 2003. Trinucleotide expansions leading to an extended poly-L-alanine segment in the poly (A) binding protein PABPN1 cause fibril formation. Protein Sci. 12: 2685-2692.
2. Kuhn, U., et al. 2003. The RNA binding domains of the nuclear poly(A)-binding protein. J. Biol. Chem. 278: 16916-16925.
3. Hino, H., et al. 2004. Myopathy phenotype in transgenic mice expressing mutated PABPN1 as a model of oculopharyngeal muscular dystrophy. Hum. Mol. Genet. 13: 181-190.
4. Davies, J.E., et al. 2005. Doxycycline attenuates and delays toxicity of the oculopharyngeal muscular dystrophy mutation in transgenic mice. Nat. Med. 11: 672-677.
5. Tavanez, J.P., et al. 2005. *In vivo* aggregation properties of the nuclear poly(A)-binding protein PABPN1. RNA 11: 752-762.

CHROMOSOMAL LOCATION

Genetic locus: PABPN1 (human) mapping to 14q11.2.

PRODUCT

PABPN1 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 µl 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 PABPN1 siRNA (h): sc-44819 and PABPN1 shRNA Plasmid (h): sc-44819-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

PABPN1 shRNA (h) Lentiviral Particles is recommended for the inhibition of PABPN1 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl 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

PABPN1 (G-17): sc-33007 is recommended as a control antibody for monitoring of PABPN1 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 PABPN1 gene expression knockdown using RT-PCR Primer: PABPN1 (h)-PR: sc-44819-PR (20 µl). 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.