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Ataxin-1 shRNA (h) Lentiviral Particles: sc-43624-V

BACKGROUND

Ataxin-1, also designated spinocerebellar ataxia type 1 protein (Sca-1), is differentially expressed and localizes to both the cytoplasm and the nucleus. Mutations in Ataxin-1 are associated with the onset of the autosomal dominant neurodegenerative disorder spinocerebellar ataxia type 1 (SCA-1), which is characterized by progressive neuronal loss in the cerebellum, muscle wasting and ataxia. In Purkinje cells, where SCA-1 is predominantly observed, Ataxin-1 has been shown to directly associate with the Purkinje-enriched leucine-rich acidic nuclear protein (LANP) and the nuclear matrix-associated protein promyelocytic leukemia protein PML. In SCA-1, Ataxin-1 is mutated to encode a polyglutamine protein that forms nuclear aggregates, which interact significantly more strongly with LANP and contribute to the pathogenesis of SCA-1.

REFERENCES

1. Banfi, S., et al. 1994. Identification and characterization of the gene causing type 1 spinocerebellar ataxia. *Nat. Genet.* 7: 513-520.
2. Burchright, E.N., et al. 1995. SCA-1 transgenic mice: a model for neurodegeneration caused by an expanded CAG trinucleotide repeat. *Cell* 82: 937-948.
3. Burchright, E.N., et al. 1997. Identification of a self-association region within the SCA1 gene product, Ataxin-1. *Hum. Mol. Genet.* 6: 513-518.
4. Skinner, P.J., et al. 1997. Ataxin-1 with an expanded glutamine tract alters nuclear matrix-associated structures. *Nature* 389: 971-974.
5. Matilla, A., et al. 1997. The cerebellar leucine-rich acidic nuclear protein interacts with Ataxin-1. *Nature* 389: 974-978.
6. Klement, I.A., et al. 1998. Ataxin-1 nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. *Cell* 95: 41-53.

CHROMOSOMAL LOCATION

Genetic locus: ATXN1 (human) mapping to 6p22.3.

PRODUCT

Ataxin-1 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 Ataxin-1 siRNA (h): sc-43624 and Ataxin-1 shRNA Plasmid (h): sc-43624-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.

APPLICATIONS

Ataxin-1 shRNA (h) Lentiviral Particles is recommended for the inhibition of Ataxin-1 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

Ataxin-1 (C-20): sc-8766 is recommended as a control antibody for monitoring of Ataxin-1 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 Ataxin-1 gene expression knockdown using RT-PCR Primer: Ataxin-1 (h)-PR: sc-43624-PR (20 μ l). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ 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.

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