SANTA CRUZ BIOTECHNOLOGY, INC.

Ataxin-3 siRNA (m): sc-40359

BACKGROUND

Autosomal dominant cerebellar ataxias are a group of neuro-degenerative disorders caused by unstable CAG repeat expansions encoding polyglutamine tracts. Proteins with long polyglutamine tracts have an increased tendency to aggregate, often forming ubiquitinated intranuclear inclusion bodies. Machado-Joseph disease (MJD)/spinocerebellar ataxia type 3 (SCA3) gene encodes Ataxin-3, which contains a polyglutamine stretch. Ataxin-3 is incorporated into most of the nuclear inclusions (NIs) and disappears from its normal cytoplasmic localization under pathological conditions in most neurons. However, in the early onset of SCA3, the association of a pathological form of Ataxin-3 with nuclear matrix alters Ataxin-3 conformation to expose the polyglutamine domain. In normal brain tissue, wild-type Ataxin-3 can also be localized within the ubiquitin-positive nuclear inclusion, the Marinesco body, under certain stressful conditions on neuronal cells such as aging and polyglutamine neurotoxicity. Cells stably expressing Ataxin-3 upregulate the mRNA levels of inflammatory response proteins, suggesting that inflammatory processes are involved in the pathogenesis of spinocerebellar ataxia type 3. Ataxin-3 binds to the N-terminus of two human homologs of the yeast DNA repair protein RAD23, HHR23A and HHR23B, which are important for nucleotide excision repair.

REFERENCES

1. Gispert, S., et al. 1993. Chromosomal assignment of the second locus for autosomal dominant cerebellar ataxia (SCA2) to chromosome 12q23-24.1 Nat. Genet. 4: 295-299.
2. Pujana, M.A., et al. 1999. Spinocerebellar ataxias in Spanish patients: genetic analysis of familial and sporadic cases. The ataxia study group. Hum. Genet. 104: 516-522.
3. Perez, M.K., et al. 1999. Ataxin-3 with an altered conformation that exposes the polyglutamine domain is associated with the nuclear matrix. Hum. Mol. Genet. 8: 2377-2385.
4. Huynh, D.P., et al. 2000. Nuclear localization or inclusion body formation of Ataxin-2 re not necessary for SCA2 pathogenesis in mouse or human. Nat. Genet. 26: 44-50.

CHROMOSOMAL LOCATION

Genetic locus: Mjd (mouse) mapping to 12 E.

PRODUCT

Ataxin-3 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 Ataxin-3 shRNA Plasmid (m): sc-40359-SH and Ataxin-3 shRNA (m) Lentiviral Particles: sc-40359-V as alternate gene silencing products.

For independent verification of Ataxin-3 (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-40359A, sc-40359B and sc-40359C.

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

Ataxin-3 siRNA (m) is recommended for the inhibition of Ataxin-3 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

Ataxin-3 (A-7): sc-398114 is recommended as a control antibody for monitoring of Ataxin-3 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 Ataxin-3 gene expression knockdown using RT-PCR Primer: Ataxin-3 (m)-PR: sc-40359-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.