

Huntingtin siRNA (m): sc-35618

BACKGROUND

Huntingtin is a protein that contains a polyglutamine region. When the number of glutamine repeats exceeds 35, the gene encodes a version of Huntingtin that leads to Huntington's disease (HD). When the polyglutamine stretch is mutated, Huntingtin acts within the nucleus to induce neurodegeneration by a cell-specific apoptotic mechanism. Loss of Huntingtin activity is unlikely to be the cause of HD, and it has been proposed that the expanded glutamine repeat region may induce an abnormal interaction between the mutant protein and other cellular proteins. Huntingtin interacts with a variety of proteins including HAP1, glyceraldehyde phosphate dehydrogenase (GAPDH), and HIP1.

REFERENCES

1. Macdonald, M.E., et al. 1993. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 72: 971-983.
2. Ambrose, C.M., et al. 1994. Structure and expression of the Huntington's disease gene: evidence against simple inactivation due to an expanded CAG repeat. *Somat. Cell Mol. Genet.* 20: 27-38.
3. Albin, R.L., et al. 1995. Genetics and molecular biology of Huntington's disease. *Trends Neurosci.* 18: 11-14.
4. Li, X.J., et al. 1995. A huntingtin-associated protein enriched in brain with implications for pathology. *Nature* 378: 398-402.
5. Burke, J.R., et al. 1996. Huntingtin and DRPLA proteins selectively interact with the enzyme GAPDH. *Nat. Med.* 2: 347-350.
6. Gusella, J.F., et al. 1996. Huntington's disease. *Cold Spring Harb. Symp. Quant. Biol.* 61: 615-626.
7. Kalchman, M.A., et al. 1997. HIP1, a human homologue of *S. cerevisiae* Sla2p, interacts with membrane-associated huntingtin in the brain. *Nat. Genet.* 16: 44-53.
8. Saudou, F., et al. 1998. Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell* 95: 55-65.

CHROMOSOMAL LOCATION

Genetic locus: Htt (mouse) mapping to 5 B2.

PRODUCT

Huntingtin 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 Huntingtin shRNA Plasmid (m): sc-35618-SH and Huntingtin shRNA (m) Lentiviral Particles: sc-35618-V as alternate gene silencing products.

For independent verification of Huntingtin (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-35618A, sc-35618B and sc-35618C.

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

Huntingtin siRNA (m) is recommended for the inhibition of Huntingtin 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 μ M in 66 μ l. 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

Huntingtin (3E10): sc-47757 is recommended as a control antibody for monitoring of Huntingtin 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG 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 Huntingtin gene expression knockdown using RT-PCR Primer: Huntingtin (m)-PR: sc-35618-PR (20 μ l, 587 bp). 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.