

HAP1 siRNA (h): sc-94188

BACKGROUND

HAP1 (Huntingtin-associated protein 1) binds to huntingtin. Huntingtin is a protein that contains a polyglutamine region and when the number of glutamine repeats exceeds 35, the gene encodes a version of Huntingtin that leads to Huntington's disease (HD). The ability of HAP1 to bind to Huntingtin is enhanced by an expanded polyglutamine repeat region. HAP1 shows neuronal localization and moves with huntingtin in nerve fibers. HAP1 is primarily expressed in brain tissue, with greater expression in the olfactory bulb and brain stem. HAP1 in rat has been shown to associate with a number of intracellular organelles. Mouse HAP1 is localized to membrane-bound organelles including large endosomes, tubulovesicular structures, and budding vesicles in neurons.

REFERENCES

1. Group THDCR. 1993. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell* 72: 971-983.
2. Li, X.J., et al. 1995. A Huntingtin-associated protein enriched in brain with implications for pathology. *Nature* 378: 398-402.
3. Gusella, J.F., et al. 1996. Huntington's disease. *Cold Spring Harb. Symp. Quant. Biol.* 61: 615-626.
4. Li, X.J., et al. 1996. Huntingtin-associated protein (HAP1): discrete neuronal localization in the brain resemble those of neuronal nitric oxide synthase. *Proc. Natl. Acad. Sci. USA* 93: 4839-4844.
5. Block-Galarza, J., et al. 1997. Fast transport and retrograde movement of Huntingtin and HAP 1 in axons. *Neuroreport* 8: 2247-2251.
6. Gutekunst, C.A., et al. 1999. Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. *J. Neurosci.* 19: 2522-2534.
7. Martin, E.J., et al. 1999. Analysis of Huntingtin associated protein 1 in mouse brain and immortalized striatal neurons. *J. Comp. Neurol.* 403: 421-430.

CHROMOSOMAL LOCATION

Genetic locus: HAP1 (human) mapping to 17q21.2.

PRODUCT

HAP1 siRNA (h) 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 HAP1 shRNA Plasmid (h): sc-94188-SH and HAP1 shRNA (h) Lentiviral Particles: sc-94188-V as alternate gene silencing products.

For independent verification of HAP1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-94188A, sc-94188B and sc-94188C.

PROTOCOLS

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

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

HAP1 siRNA (h) is recommended for the inhibition of HAP1 expression in human 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

HAP1 (C-3): sc-166245 is recommended as a control antibody for monitoring of HAP1 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 HAP1 gene expression knockdown using RT-PCR Primer: HAP1 (h)-PR: sc-94188-PR (20 μ l). 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.