SANTA CRUZ BIOTECHNOLOGY, INC.

HAP40 siRNA (h): sc-75225

BACKGROUND

The Huntingtin protein contains a polyglutamine region, which leads to Huntingtin's disease (HD) when the number of glutamine repeats exceeds 35. The mutated Huntingtin protein acts within the nucleus to induce neurodegeneration by a cell-specific apoptotic mechanism. The loss of activity of the Huntingtin protein may be contributed to abnormal interactions between the mutant protein and other associated cellular proteins. Huntingtin interacts with a variety of proteins including HAP1, glyceraldehyde phosphate dehydrogenase (GAPDH), HAP40, Rab5 and HIP1. HAP40 mediates the recruitment of Huntingtin by Rab5 onto early endosomes. Specifically, this complex regulates endosome motility, which may be a key event of the pathogenetic process leading to neurodegeneration in HD.

REFERENCES

1. 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.
2. Albin, R.L., et al. 1995. Genetics and molecular biology of Huntington's disease. Trends Neurosci. 18: 11-14.
3. Gusella, J.F., et al. 1996. Huntington's disease. Cold Spring Harb. Symp. Quant. Biol. 61: 615-626.
4. 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.
5. Peters, M.F., et al. 2001. Isolation of a 40-kDa Huntingtin-associated protein. J. Biol. Chem. 276: 3188-3194.
6. Pal, A., et al. 2006. Huntingtin-HAP40 complex is a novel Rab5 effector that regulates early endosome motility and is up-regulated in Huntington's disease. J. Cell Biol. 172: 605-618.
7. Ravikumar, B., et al. 2008. Rab5 modulates aggregation and toxicity of mutant huntingtin through macroautophagy in cell and fly models of Huntington disease. J. Cell Sci. 121: 1649-1660.

CHROMOSOMAL LOCATION

Genetic locus: F8A1 (human) mapping to Xq28.

PRODUCT

HAP40 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HAP40 shRNA Plasmid (h): sc-75225-SH and HAP40 shRNA (h) Lentiviral Particles: sc-75225-V as alternate gene silencing products.

For independent verification of HAP40 (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-75225A, sc-75225B and sc-75225C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

HAP40 siRNA (h) is recommended for the inhibition of HAP40 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 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor HAP40 gene expression knockdown using RT-PCR Primer: HAP40 (h)-PR: sc-75225-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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