

HuD siRNA (h): sc-37835

BACKGROUND

The Elav-like genes encode for a family of RNA-binding proteins. Elav, a *Drosophila* protein and the first described member, is expressed immediately after neuroblastic differentiation into neurons and is necessary for neuronal differentiation and maintenance. Several mammalian Elav-like proteins, designated HuC, HuD and Hel-N1, are also expressed in postmitotic neurons. An additional mammalian homolog, HuR, which is also designated HuA, is ubiquitously expressed and is also overexpressed in a wide variety of tumors. Characteristically, these homologs all contain three RNA recognition motifs (RRM) and they specifically bind to AU-rich elements (ARE) in the 3'-untranslated region of mRNAs transcripts. ARE sites target mRNA for rapid degradation and thereby regulate the expression levels of genes involved in cell growth and differentiation. When Elav-like proteins associate with these ARE sites this degradation is inhibited, leading to an increased stability of the corresponding transcript. Elav proteins function within the nucleus, and they are shuttled between the nucleus and cytoplasm by a nuclear export signal, which is a regulatory feature of the Elav-like proteins as it limits their accessibility to ARE sites.

REFERENCES

1. Chagnovich, D., et al. 1996. Differential activity of ELAV-like RNA-binding proteins in human neuroblastoma. *J. Biol. Chem.* 271: 33587-33591.
2. Wakamatsu, Y., et al. 1997 Sequential expression and role of Hu RNA-binding proteins during neurogenesis. *Development* 124: 3449-3460.
3. King, P. 1997. Differential expression of the neuroendocrine genes Hel-N1 and HuD in small-cell lung carcinoma: evidence for down-regulation of HuD in the variant phenotype. *Int. J. Cancer* 74: 378-382.

CHROMOSOMAL LOCATION

Genetic locus: ELAVL4 (human) mapping to 1p33.

PRODUCT

HuD 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 HuD shRNA Plasmid (h): sc-37835-SH and HuD shRNA (h) Lentiviral Particles: sc-37835-V as alternate gene silencing products.

For independent verification of HuD (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-37835A, sc-37835B and sc-37835C.

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

HuD siRNA (h) is recommended for the inhibition of HuD 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

HuD (H-9): sc-48421 is recommended as a control antibody for monitoring of HuD 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 HuD gene expression knockdown using RT-PCR Primer: HuD (h)-PR: sc-37835-PR (20 μ l, 582 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Abdelmohsen, K., et al. 2010. miR-375 inhibits differentiation of neurites by lowering HuD levels. *Mol. Cell. Biol.* 30: 4197-4210.
2. Lim, C.S. and Alkon, D.L. 2014. PKC ϵ promotes HuD-mediated neprilysin mRNA stability and enhances neprilysin-induced A β degradation in brain neurons. *PLoS ONE* 9: e97756.

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.