

UCH-L3 siRNA (h): sc-42306

BACKGROUND

UCH-L1 (ubiquitin C-terminal hydrolase) is a member of a gene family whose products hydrolyze small C-terminal adducts of ubiquitin to generate the ubiquitin monomer. Expression of UCH-L1 is highly specific to neurons and to cells of the diffuse neuroendocrine system and their tumors. UCH-L1 is expressed in brain neurons. Examination of specific brain regions reveals expression in all areas tested, particularly in the substantia nigra. UCH-L1 represents 1 to 2% of total soluble brain protein. Its occurrence in Lewy bodies and its function in the proteasome pathway make it a compelling candidate gene in Parkinson disease. The gene which encodes UCH-L1 maps to human chromosome 4p14. The 230 amino acid human UCH-L3 protein is 54% identical to that of UCH-L1. UCH-L3 is the predominant thiol protease and has high-affinity binding sites for ubiquitin.

REFERENCES

1. Doran, J.F., Jackson, P., Kynoch, P. and Thompson, R.J. 1983. Isolation of PGP 9.5, a new human neurone-specific protein detected by high resolution two-dimensional electrophoresis. *J. Neurochem.* 40: 1542-1547.
2. Wilkinson, K.D., Lee, K.M., Deshpande, S., Duerksen-Hughes, P., Boss, J.M. and Pohl, J. 1989. The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. *Science* 246: 670-672.
3. Mayer, A.N. and Wilkinson, K.D. 1989. Detection, resolution and nomenclature of multiple ubiquitin carboxyl-terminal esterases from bovine calf thymus. *Biochemistry* 28: 166-172.
4. Edwards, Y.H., Fox, M.F., Povey, S., Hinks, L.J., Day, I.N.M. and Thompson, R.J. 1991. The gene for human neuron specific ubiquitin C-terminal hydrolase maps to chromosome 4p14. *Cytogenet. Cell Genet.* 58: 1886-1887.
5. Leroy, E., Boyer, R. and Polymeropoulos, M.H. 1998. Intron-exon structure of ubiquitin C-terminal hydrolase-L1. *DNA Res.* 5: 397-400.

CHROMOSOMAL LOCATION

Genetic locus: UCHL3 (human) mapping to 13q22.2.

PRODUCT

UCH-L3 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 UCH-L3 shRNA Plasmid (h): sc-42306-SH and UCH-L3 shRNA (h) Lentiviral Particles: sc-42306-V as alternate gene silencing products.

For independent verification of UCH-L3 (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-42306A, sc-42306B and sc-42306C.

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

UCH-L3 siRNA (h) is recommended for the inhibition of UCH-L3 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

UCH-L3 (ZE-17): sc-100340 is recommended as a control antibody for monitoring of UCH-L3 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 UCH-L3 gene expression knockdown using RT-PCR Primer: UCH-L3 (h)-PR: sc-42306-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.