SANTA CRUZ BIOTECHNOLOGY, INC.

Dorfin siRNA (h): sc-62224

BACKGROUND

Dorfin (double ring-finger protein), also known as RNF19A or RNF19, is a multi-pass membrane, RING-IBR type, E3 ubiquitin-protein ligase. It is widely expressed with highest levels found in heart and ubiquitous expression throughout the central nervous system. Dorfin functions by accepting ubiquitin in the form of a thioester from UBCH7 and UBC8 and then transferring it to the targeted substrates. Dorfin is responsible for ubiquitylating synphilin-1, CaSR and mutant variants of SOD-1, a protein at fault for familial ALS (amyotrophic lateral sclerosis). Dorfin physically interacts with VCP (valosin-containing protein) via its C-terminus. Together these two proteins are associated with the formation of ubiquitylated inclusions (UBIs) that characterize many neurodegenerative disorders, such as Parkinson's disease and ALS. This association with UBIs suggests that Dorfin plays an important role in the disease process.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607119. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
2. Hishikawa, N., et al. 2003. Dorfin localizes to the ubiquitylated inclusions in Parkinson's disease, dementia with Lewy bodies, multiple system atrophy, and amyotrophic lateral sclerosis. Am. J. Pathol. 163: 609-619.
3. Ito, T., et al. 2003. Dorfin localizes to Lewy bodies and ubiquitylates synphilin-1. J. Biol. Chem. 278: 29106-29114.
4. Ishigaki, S., et al. 2004. Physical and functional interaction between Dorfin and Valosin-containing protein that are colocalized in ubiquitylated inclusions in neurodegenerative disorders. J. Biol. Chem. 279: 51376-51385.
5. Takeuchi, H., et al. 2004. Dorfin prevents cell death by reducing mitochondrial localizing mutant superoxide dismutase 1 in a neuronal cell model of familial amyotrophic lateral sclerosis. J. Neurochem. 89: 64-72.
6. Huang, Y., et al. 2006. Calcium-sensing receptor ubiquitination and degradation mediated by the E3 ubiquitin ligase dorfin. J. Biol. Chem. 281: 11610-11617.

CHROMOSOMAL LOCATION

Genetic locus: RNF19A (human) mapping to 8q22.2.

PRODUCT

Dorfin 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 Dorfin shRNA Plasmid (h): sc-62224-SH and Dorfin shRNA (h) Lentiviral Particles: sc-62224-V as alternate gene silencing products.

For independent verification of Dorfin (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-62224A, sc-62224B and sc-62224C.

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

Dorfin siRNA (h) is recommended for the inhibition of Dorfin 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 Dorfin gene expression knockdown using RT-PCR Primer: Dorfin (h)-PR: sc-62224-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.