

UBC8 siRNA (m): sc-41686

BACKGROUND

Ubiquitin is an abundant, highly conserved protein found in all eukaryotic cells, either free or covalently attached to cellular proteins. The primary function of ubiquitin in mammalian systems is to clear abnormal, foreign, and improperly folded proteins by targeting them for proteasome degradation. Ubiquitin conjugating enzyme 8 (UBC8) is an E2 enzyme involved in the ubiquitin pathway for protein degradation. Like other E2 enzymes, UBC8 forms a thioester bond with ubiquitin in an E1-dependent manner. UBC8 binds to the human homolog of *Drosophila ariadne* (HHARI) and UBC7-associated protein (H7-AP1) as well as double ring-finger protein (Dorfin). UBC8 is enriched in the central nervous system and interacts with Parkin, a RING-finger-containing protein implicated in the pathogenesis of familial Parkinson's disease. Parkin shares sequence homology with other UBC8 binding proteins such as HHARI and H7-AP1.

REFERENCES

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2. Ciechanover, A., et al. 1994. The ubiquitin-mediated proteolytic pathway: mechanisms of recognition of the proteolytic substrate and involvement in the degradation of native cellular proteins. *FASEB J.* 8: 182-191.
3. Hochstrasser, M. 1995. Ubiquitin, proteasomes and the regulation of intracellular protein degradation. *Curr. Opin. Cell Biol.* 7: 215-223.
4. Kimura, M., et al. 1997. cDNA cloning, characterization, and chromosome mapping of UBE2E2 encoding a human ubiquitin-conjugating E2 enzyme. *Cytogenet. Cell Genet.* 78: 107-111.
5. Moynihan, T.P., et al. 1999. The ubiquitin-conjugating enzymes UbcH7 and UbcH8 interact with RING finger/IBR motif-containing domains of HHARI and H7-AP1. *J. Biol. Chem.* 274: 30963-30968.
6. Tan, N.G., et al. 2000. Characterisation of the human and mouse orthologues of the *Drosophila ariadne* gene. *Cytogenet. Cell Genet.* 90: 242-245.
7. Zhang, Y., et al. 2000. Parkin functions as an E2-dependent ubiquitin-protein ligase and promotes the degradation of the synaptic vesicle-associated protein, CDCrel-1. *Proc. Natl. Acad. Sci. USA* 97: 13354-13359.

CHROMOSOMAL LOCATION

Genetic locus: Ube2l6 (mouse) mapping to 2 D.

PRODUCT

UBC8 siRNA (m) 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 UBC8 shRNA Plasmid (m): sc-41686-SH and UBC8 shRNA (m) Lentiviral Particles: sc-41686-V as alternate gene silencing products.

For independent verification of UBC8 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41686A, sc-41686B and sc-41686C.

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

UBC8 siRNA (m) is recommended for the inhibition of UBC8 expression in mouse 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

UBC8 (E-5): sc-166276 is recommended as a control antibody for monitoring of UBC8 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 UBC8 gene expression knockdown using RT-PCR Primer: UBC8 (m)-PR: sc-41686-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.

PROTOCOLS

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