

UFD2 siRNA (h): sc-45980

BACKGROUND

UFD2, also designated ubiquitin conjugation factor E4 (UBE4B), binds to the ubiquitin moieties of preformed conjugates and catalyzes ubiquitin chain assembly in conjunction with E1, E2 and E3. During apoptosis, UFD2 is proteolytically cleaved at Asp 123 by caspase-6 and granzyme B, and is cleaved with approximately 10-fold less efficiency at Asp 109 by caspase-3 and caspase-7. In yeast, E4 activity is linked to cell survival under stress conditions, indicating that eukaryotes use E4-dependent proteolysis pathways for multiple cellular functions. In mammals, highest expression of UFD2 is in ovary, testis, heart and skeletal muscle.

REFERENCES

1. Koegl, M., et al. 1999. A novel ubiquitination factor, E4, is involved in multiubiquitin chain assembly. *Cell* 96: 635-644.
2. Conforti, L., et al. 2000. A UFD2/D4Cole1e chimeric protein and overexpression of Rbp7 in the slow Wallerian degeneration (WldS) mouse. *Proc. Natl. Acad. Sci. USA* 97: 11377-11382.
3. Krona, C., et al. 2003. Screening for gene mutations in a 500 kb neuroblastoma tumor suppressor candidate region in chromosome 1p; mutation and stage-specific expression in UBE4B/UFD2. *Oncogene* 22: 2343-2351.
4. Spinette, S., et al. 2004. UFD2, a novel autoantigen in scleroderma, regulates sister chromatid separation. *Cell Cycle* 3: 1638-1644.
5. Saeki, Y., et al. 2004. Definitive evidence for UFD2-catalyzed elongation of the ubiquitin chain through Lys 48 linkage. *Biochem. Biophys. Res. Commun.* 320: 840-845.
6. Bazirgan, O.A., et al. 2005. Cdc48-UFD2-Rad23: the road less ubiquitinated? *Nat. Cell. Biol.* 7: 207-209.
7. SWISS-PROT/TrEMBL (O95155). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: UBE4B (human) mapping to 1p36.22.

PRODUCT

UFD2 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 UFD2 shRNA Plasmid (h): sc-45980-SH and UFD2 shRNA (h) Lentiviral Particles: sc-45980-V as alternate gene silencing products.

For independent verification of UFD2 (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-45980A, sc-45980B and sc-45980C.

PROTOCOLS

See our web site at www.scbt.com or our catalog 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

UFD2 siRNA (h) is recommended for the inhibition of UFD2 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

UFD2 (C-1): sc-377072 is recommended as a control antibody for monitoring of UFD2 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor UFD2 gene expression knockdown using RT-PCR Primer: UFD2 (h)-PR: sc-45980-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.