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UBC2 siRNA (m): sc-41678

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. In *Saccharomyces cerevisiae*, ubiquitin-like proteins include Rub1, Ula1, Uba3, Smt3, Ubc2, Ubc12 and Ubc9. Rub1 shares 53% homology with ubiquitin and requires activation via Ula1, Uba3 and Ubc12 in order to conjugate to substrates directed to different proteolytic systems. Smt3, which is similar to mammalian SUMO-1, requires Ubc9 for conjugation to other proteins. Skp1 connects cell cycle regulators to the ubiquitin proteolysis machinery. Hrt1 is an essential subunit of Skp1p-cullin-F-box (SCF) complexes, which are necessary for the degradation of various regulatory proteins. Ubc13 forms a complex with Mms2 that is involved in the error-free DNA postreplication repair (PRR) pathway.

REFERENCES

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3. Hochstrasser, M. 1995. Ubiquitin, proteasomes and the regulation of intracellular protein degradation. *Curr. Opin. Cell Biol.* 7: 215-223.
4. Bai, C., et al. 1996. SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. *Cell* 86: 263-274.
5. Liakopoulos, D., et al. 1998. A novel protein modification pathway related to the ubiquitin system. *EMBO J.* 17: 2208-2214.
6. Schwarz, S.E., et al. 1998. The ubiquitin-like proteins SMT3 and SUMO-1 are conjugated by the UBC9 E2 enzyme. *Proc. Natl. Acad. Sci. USA* 95: 560-564.
7. Gong, L., et al. 1999. Identification of the activating and conjugating enzymes of the NEDD8 conjugation pathway. *J. Biol. Chem.* 274: 12036-12042.

CHROMOSOMAL LOCATION

Genetic locus: Ube2a (mouse) mapping to X A3.3.

PRODUCT

UBC2 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 UBC2 shRNA Plasmid (m): sc-41678-SH and UBC2 shRNA (m) Lentiviral Particles: sc-41678-V as alternate gene silencing products.

For independent verification of UBC2 (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-41678A, sc-41678B and sc-41678C.

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

UBC2 siRNA (m) is recommended for the inhibition of UBC2 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

UBC2 (2782C5): sc-81419 is recommended as a control antibody for monitoring of UBC2 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 UBC2 gene expression knockdown using RT-PCR Primer: UBC2 (m)-PR: sc-41678-PR (20 μ l, 362 bp). 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.